ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ

ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΑΤΤΙΚΟΝ ΠΑΝΕΠΙΣΤΗΜΙΑΚΟ ΝΟΣΟΚΟΜΕΙΟ
Δ' ΠΑΝΕΠΙΣΤΗΜΙΑΚΗ ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ
ΔΙΕΥΘΥΝΤΗΣ: ΔΗΜΗΤΡΙΟΣ ΜΠΟΥΜΠΑΣ

ΓΕΝΩΜΙΚΗ ΑΝΑΛΥΣΗ ΣΤΟ ΣΥΣΤΗΜΑΤΙΚΟ ΕΡΥΘΗΜΑΤΩΔΗ ΛΥΚΟ: ΜΟΡΙΑΚΟΙ ΜΗΧΑΝΙΣΜΟΙ ΣΤΗΝ ΙΣΤΙΚΗ ΒΛΑΒΗ ΚΑΙ ΤΗΝ ΑΠΟΚΡΙΣΗ

ΘΕΟΔΩΡΑ ΜΑΝΩΛΑΚΟΥ

ΒΙΟΤΕΧΝΟΛΟΓΟΣ, MSc

ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

ΝΟΕΜΒΡΙΟΣ 2022

ΑΘΗΝΑ

NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

SCHOOL OF HEALTH SCIENCES

SCHOOL OF MEDICINE

ATTIKON UNIVERSITY HOSPITAL

4TH DEPARTMENT OF INTERNAL MEDICINE
HEAD: DIMITRIOS BOUMPAS

GENOMIC ANALYSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS: MOLECULAR MECHANISMS FOR TISSUE INJURY AND RESPONSE

THEODORA MANOLAKOU

BIOTECHNOLOGIST, MSc

DOCTORAL THESIS

NOVEMBER 2022

ATHENS

Ημερομηνία αίτησης: 03-09-2018

Ημερομηνία ορισμού τριμελούς Συμβουλευτικής Επιτροπής: 18-12-2018

Μέλη τριμελούς Συμβουλευτικής Επιτροπής:

Δημήτριος Μπούμπας (Επιβλέπων Καθηγητής)

Γεώργιος Μπερτσιάς

Παναγιώτης Πολίτης

Ημερομηνία ορισμού θέματος: 18-03-2019

Ημερομηνία καταθέσεως της διδακτορικής διατριβής: 14-11-2023

Πρόεδρος Ιατρικής Σχολής:

Καθηγητής Γεράσιμος Δ. Σιάσος

Επταμελής Εξεταστική Επιτροπή:

1. Δημήτριος Μπούμπας (Επιβλέπων Καθηγητής), Καθηγητής, Ιατρική Σχολή, ΕΚΠΑ
2. Γεώργιος Μπερτσιάς, Αναπληρωτής Καθηγητής, Ιατρική Σχολή, Πανεπιστήμιο Κρήτης
3. Παναγιώτης Βεργίνης, Αναπληρωτής Καθηγητής, Ιατρική Σχολή, Πανεπιστήμιο Κρήτης
4. Θέμις Αλισσάφη, Επίκουρη Καθηγήτρια, Ιατρική Σχολή, ΕΚΠΑ
5. Βασιλική Λαμπροπούλου, Επίκουρη Καθηγήτρια, Ιατρική Σχολή, ΕΚΠΑ
6. Αντώνης Φανουριάκης, Επίκουρος Καθηγητής, Ιατρική Σχολή, ΕΚΠΑ
7. Παναγιώτης Πολίτης, Ερευνητής Β', Κέντρο Βασικής Έρευνας, Ίδρυμα Ιατροβιολογικών Ερευνών Ακαδημίας Αθηνών

Acknowledgements

The present study was performed at the Biomedical Research Foundation of the Academy of Athens in collaboration with the 4th Pathology Clinic in Attikon University Hospital under the supervision of Dr. Boumpas. I express the warmest thanks for the assignment of this subject and the advice during the course of my doctoral thesis. I am especially thankful to Dr. Panayotis Verginis for his valuable help and inspiring discussions. I had also great help from Dr. Themis Alissafi, Dr. Panagiotis Politis and Dr. Aristidis Charonis who offered really interesting and insightful observations on my experimental data.

I would like to thank Dr. Angelos Banos, Dr. Manolis Giallitakis, Dr. Anastasia Filia, Dr. Katerina Hatzioannou, Dr. Dimitris Gkikas, Dr. Vicky Lampropoulou, Dr. Aristidis Charonis and Dr. George Bertsias for sharing their expertise and the invaluable discussions we had. I would also like to thank my colleagues Maria Grigoriou, Athina Varveri, Miranta Papadopoulou, Athina Boumpa, Stavros Doumas, Eirini Stergioti, Eleni Zervopoulou, George Sentis, Panagiotis Garatziotis and Alex Grivas. Even though we are good colleagues, I consider us good friends. I could not be more grateful to the clinicians' team Dr. Antonis Fanourakis, Dr. Antigone Pieta, Dr. Noemin Kapsala and. Dr. Sophia Flouda for providing invaluable human samples.

I owe thanks to a very special person, Dionysis Nikolopoulos, my colleague and partner, for his never- ending encouragement, understanding and for always being there for me. Finally, I could not forget my family that has always been an inspiration to pursue my studies, and my friends, Athina Kalamata and Vicky Patrianakou. Without their help and their support, the completion of my thesis would not be feasible.

Contents

ABSTRACT

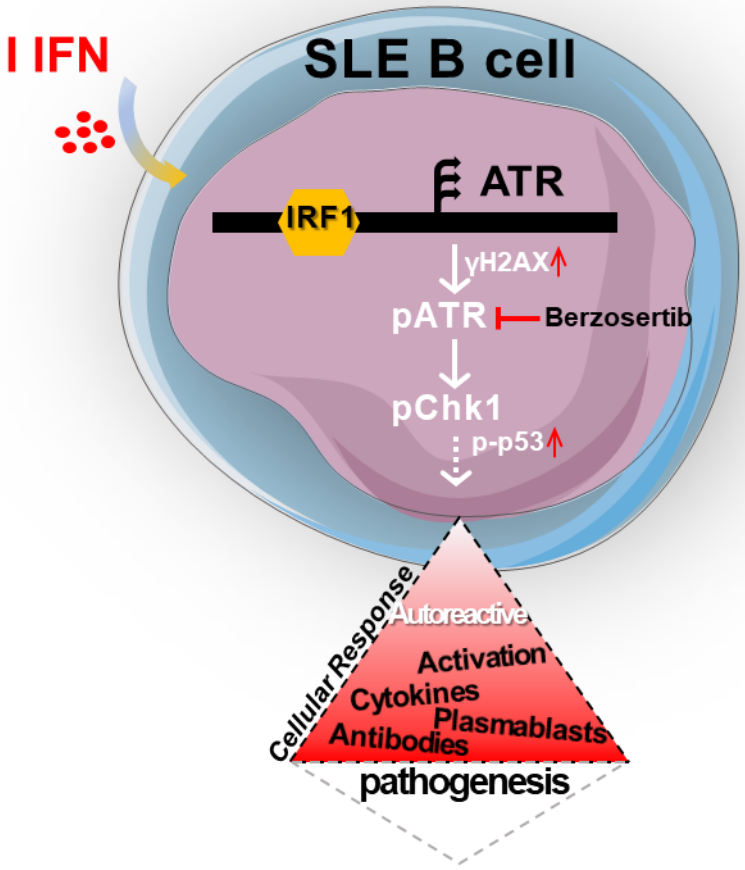
Systemic lupus erythematosus (SLE) is a heterogenous autoimmune and potentially severe disease. In SLE, an interplay between environmental and genetic factors leads to perturbation of complex biological networks that culminate in immune dysregulation and diverse clinical phenotypes. B cells are the main orchestrators of autoimmune responses in patients with SLE. However broad-based B-cell directed therapies show modest efficacy, while blunting humoral immune responses to vaccines and inducing immunosuppression, underscoring the need for development of more effective therapies targeting the pathogenic B-cell clones. Under steady state, B cells are prone to enriched DNA damage response (DDR) since DDR is co-opted in antibody diversification. Although patients with SLE demonstrate increased levels of DNA damage in genes required for efficient DDR, the role of the DDR in B-cells pathogenicity remains elusive. Furthermore, analysis in disease-involved tissues such as lesional skin indicated higher levels of DNA damage when compared to non-lesional or healthy skin. To this end, our transcriptomic analysis in whole blood cells of patients with SLE compared to healthy subjects, highlights the aberrant expression profile of DDR pathways. This was associated via microscopy studies with enhanced DDR in peripheral B cells of both patients with SLE and murine SLE model. In this direction, utilizing transcriptomic, proteomic and immunofluorescence studies, this thesis provides evidence for enhanced activation of the ATR/Chk1 DDR pathway in B cells of patients with active SLE disease. Treatment of B cells with type I IFN, a key driver of immunity in SLE, induced expression of ATR via direct binding of interferon regulatory factor 1 (IRF1) to its gene promoter. Pharmacologic targeting of ATR in B cells attenuated their immunogenic profile, including proinflammatory cytokine secretion, plasmablast formation and antibody production. Together, these findings identify the ATR-mediated DDR axis as the orchestrator of the type-I IFN-mediated B cell responses in SLE and as a potential novel therapeutic target.

ΠΕΡΙΛΗΨΗ

Ο Συστηματικός ερυθηματώδης λύκος (ΣΕΛ) είναι μια ετερογενής αυτοάνοση και δυνητικά σοβαρή νόσος. Στον ΣΕΛ, η αλληλεπίδραση μεταξύ περιβαλλοντικών και γενετικών παραγόντων οδηγεί στη διαταραχή πολύπλοκων βιολογικών δικτύων καταλήγοντας στην απορρύθμιση του ανοσοποιητικού συστήματος και σε διαφορετικούς κλινικούς φαινοτύπους. Τα Β κύτταρα είναι οι κύριοι ενορχηστρωτές των αυτοάνοσων αποκρίσεων στους ασθενείς με ΣΕΛ. Ωστόσο, οι ευρέως χρησιμοπούμενες θεραπείες που στοχεύουν τα Β-κύτταρα δείχνουν μέτρια αποτελεσματικότητα, ενώ αμβλύνουν τις χυμικές ανοσολογικές αποκρίσεις στα εμβόλια και προκαλούν ανοσοκαταστολή, αναδεικνύοντας την ανάγκη για ανάπτυξη πιο αποτελεσματικών θεραπειών που στοχεύουν τους παθογόνους κλώνους. Σε φυσιολογικές καταστάσεις, τα Β κύτταρα χρησιμοποιούν μηχανισμούς απόκρισης προς τις βλάβες του DNA (DNA damage response, DDR) για τη δημιουργία αντισωμάτων καθώς αυτά απαιτούν γενετικό ανασυνδιασμό. Αν και οι ασθενείς με ΣΕΛ εμφανίζουν αυξημένες βλάβες στα γονίδια που απαιτούνται για την αποτελεσματικότητα του DDR, ο ρόλος του DDR στην παθογένεια των Β κυττάρων παραμένει ασαφής. Επιπλέον, η ανάλυση ιστών που προσβάλλονται από τον ΣΕΛ, όπως το δέρμα, έδειξε υψηλότερα επίπεδα DDR σε σύγκριση με υγιές δέρμα. Προς αυτήν την κατεύθυνση, η μεταγραφική ανάλυση σε ολικά κύτταρα περιφερικού αίματος ασθενών με ΣΕΛ σε σύγκριση με υγιή άτομα, αναδεικνύει τους DDR μοριακούς μηχανισμούς. Μέσω μελετών μικροσκοπίας, το προφίλ αυτό συσχετίστηκε με ενισχυμένο DDR σε Β κύτταρα τόσο ασθενών με ΣΕΛ όσο και μοντέλου ποντικού με ΣΕΛ. Χρησιμοποιώντας μεταγραφικές, πρωτεομικές και μελέτες ανοσοφθορισμού, η παρούσα διατριβή αποκαλύπτει την ενεργοποίηση του μοριακού DDR μονοπατιού ATR/Chk1 σε Β κύτταρα ασθενών με ΣΕΛ με ενεργό νόσο. Χορήγηση ιντερφερόνης τύπου Ι (IFN I) στα Β κύτταρα, βασικός μεσολαβητής της ανοσίας στον ΣΕΛ, προκάλεσε έκφραση του ATR μέσω άμεσης σύνδεσης του ρυθμιστικού παράγοντα 1 ιντερφερόνης (IRF1) στον υποκινητή του γονιδίου του. Η φαρμακολογική στόχευση του ATR, χρησιμοποιώντας έναν ειδικό αναστολέα (VE-822, εμπορικά γνωστό ως berzosertib), μείωσε το ανοσογονικό προφίλ των Β κυττάρων, συμπεριλαμβανομένης της έκκρισης προφλεγμονώδων κυτοκινών, του σχηματισμού πλασμαβλαστών και της παραγωγής αντισωμάτων. Συνολικά αυτά τα

ευρήματα υποδηλώνουν ότι η μοριακή σχέση IRF1-ATR είναι σημαντική για τη δραστηριότητα των Β κυττάρων στο ΣΕΛ. Συμπερασματικά, η παρούσα διατριβή αναδεικνύει τον παθογενετικό ρόλο του DDR με τη μεσολάβηση του ATR στις αποκρίσεις των Β κυττάρων που διαμεσολαβούνται από IFN I στον ΣΕΛ, και προτείνει τον ATR ως πιθανό νέο θεραπευτικό στόχο.

GRAPHICAL ABSTRACT



Outline

Teaser: DNA damage response as a critical regulator of autoimmune B cell responses in SLE disease

B cells in SLE demonstrate an autoreactive phenotype characterized by excessive activation, plasmablast formation, cytokines and antibodies production driving pathogenesis. This thesis provides evidence that this phenotype is triggered by ATR-mediated DNA damage response (DDR) through direct interaction with IRF1, an important modulator of the type I IFN signature in SLE. Pharmacological targeting of ATR, using a specific inhibitor (VE-822, marketed as berzosertib), attenuated B-cell immunogenic profile, paving the way for novel therapeutic targets via DDR manipulation.

INTRODUCTION

Autoimmune diseases

Autoimmune diseases (AD) encompass a wide group of clinical syndromes caused by aberrant activation of autoreactive immune cells in the absence of ongoing infection or other discernible cause (1). Although individually rare, collectively they affect up to 5% of the population in Western countries especially women (2). These diseases run into families with members of the same family being affected with the same or other autoimmune diseases, or occasionally the same patient being affected by more than one AD. An interplay of environmental, genetic and epigenetic factors leading to perturbation of complex biological networks has been well documented with a steady increase in prevalence after WWII that cannot be merely attributed to genetic factors (3).

Systemic lupus erythematosus: the prototypic systemic autoimmune disease

Pathogenesis

Systemic Lupus Erythematosus (SLE) is the prototypic systemic, chronic autoimmune disease that manifests a wide range of clinical and molecular abnormalities (4,5). The etiology remains elusive although several components of the innate and adaptive immunity and various factors (sex, environment, genes) contribute to the disease **(Fig. 1).** In lupus, α-interferon (IFNα)-driven immunologic alterations culminate into persistent self-directed immune responses against autologous nucleic acids, mimicking a sustained antiviral response. Excessive and intractable tissue damage caused mainly via the adaptive immune response comprised of autoantibodies or immune-complex depositions, affects the skin, kidneys, heart, vessels, central nervous system, lungs, muscles and joints leading to significant morbidity and increased mortality **(Fig. 2)**. Key cellular pathways in lupus include a) autoantibody production several years prior to the clinical onset of disease; b) increased cellular apoptosis and defective clearance of endogenous apoptotic material, c) self-nucleic acid recognition and production of IFNα; and d) activation of monocytes, neutrophils, monocytes, B and T cells (6,7) **(Fig. 1, 2)**. These pathways have been reiterated by genome-wide

association studies (GWAS) which have demonstrated the involvement of genes related to immune responses, endothelial function and tissue response to injury (8,9).

Despite advances in the pathogenesis and treatment, several unmet needs exist in SLE: Flares are common (approximately 0.3 flares per year) even in well controlled patients, posing the risk of additional damage. Despite the introduction of the first targeted biological agent in SLE (10), a sizable proportion of patients will be unresponsive to existing treatments, highlighting the need for novel, targeted therapies based on the underlying immune aberrancies. Importantly, comorbidities especially accelerated atherosclerosis and a risk for infection –above and beyond the risk of immunosuppression- are responsible for most deaths in lupus (4,5).

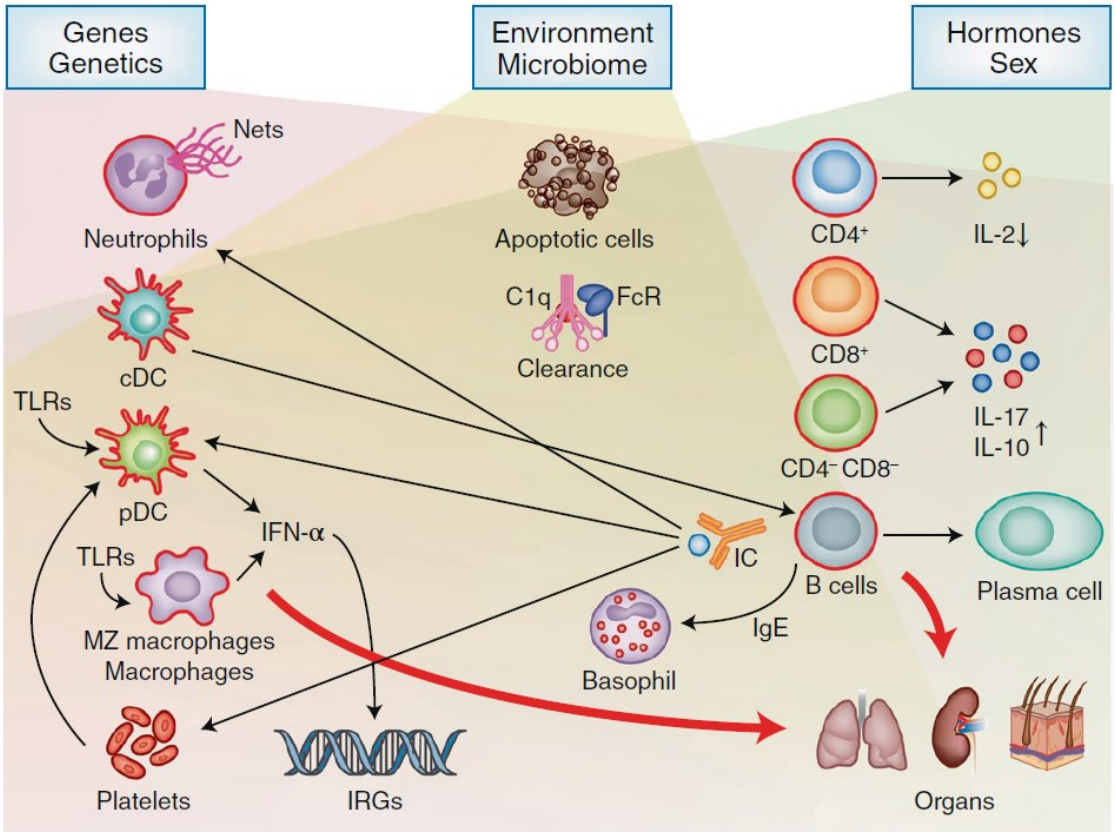


Figure 1. Pathogenic features of SLE. Genetics, environment and sex affect both innate and adaptive immunity in various ways and culminate in organ damage from Tsokos (11).

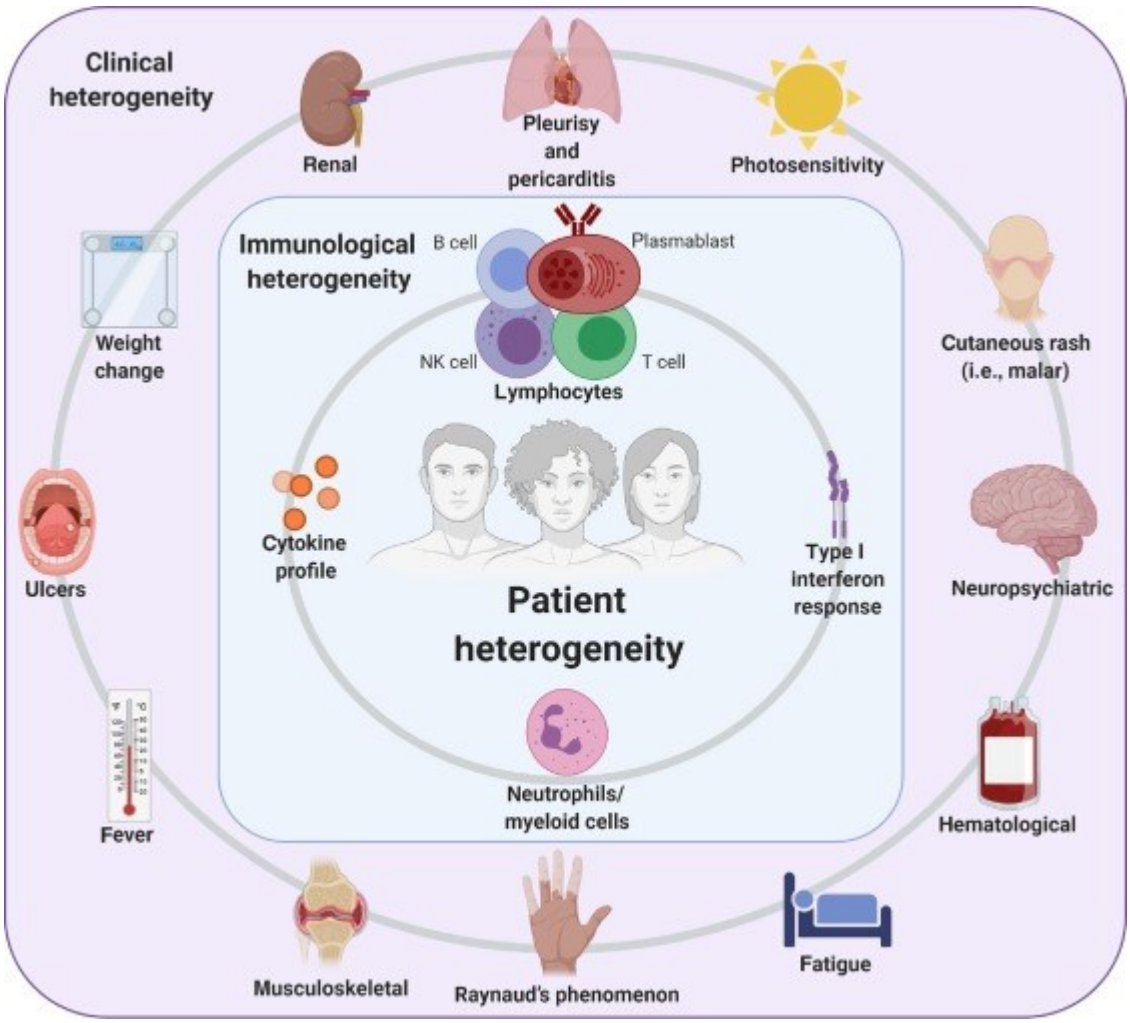


Figure 2. Clinical heterogeneity is SLE patients. From Allen et al (12).

The central role of B cells in SLE pathogenesis

The main characteristic of SLE is the production of autoantibodies, which places B cells centrally in SLE etiology. In SLE, autoreactive B cells fail during the tolerance checkpoints due to toll-like receptor (TLR), cytokine and/ or co-receptor malfunction thereby leading to the expansion of pathogenic B cells (13). Moreover, GWAS have demonstrated a striking concentration of disease-susceptibility alleles in the B cell antigen receptor (BCR) signaling and B cell costimulatory pathways in SLE.

B-cell aberrancies are critically involved in all levels of SLE pathology, contributing to immune deregulation (13–15). More specifically, B cell key pathogenic features in SLE include a) a hyperactive profile with increased antigen-presentation capability, b) expanded populations of plasmablasts, memory, double-negative memory and transitional cells, c) aberrancies in both pro-and anti- inflammatory cytokines secretion d) augmented secretion of pathogenic autoantibodies targeting nuclear components (anti-double stranded DNA: anti-dsDNA) and d) priming autoreactive T cells (**Fig. 3**). This aberrant B-cell function in SLE is affected by type I IFN correlating with an IFN- molecular signaling present especially in patients with moderate to severe lupus (16,17).

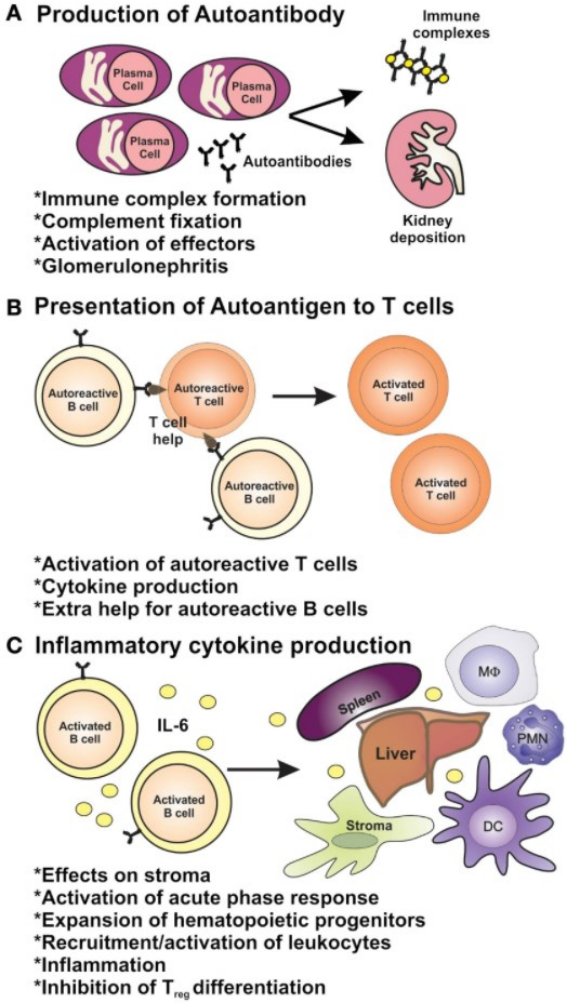
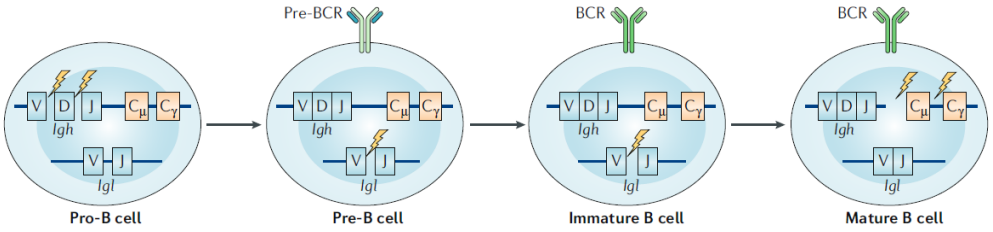


Figure 3. Key roles of B cells in SLE pathogenesis. They include **(A)** their ability to produce autoantibodies, **(B)** their role as antigen-presenting cells and **(C)** their ability to produce cytokines. From Gottschalk et al (18).

Advances in our understanding of the pathogenesis of SLE have corroborated the importance of B-cell-targeted therapies –some being already approved for the treatment of SLE (19,20). Current B-cell-directed strategies include targeting of B-cell

surface antigens, cytokines that promote B-cell growth and functions, and B- and T-cell interactions.

Despite the fact that B cells are central in SLE disease etiology, the mechanisms driving their deregulation are only partly understood. Importantly, many SLE patients are not responsive to the current B-cell-targeted therapies (21,22). Thus, advances in B-cell immunology may facilitate the development of novel, safer and more effective therapies that target specific aspects of B-cell biology. Interestingly, physiological activation and development of B cells depends on normal DNA damage response (DDR) processes **(Fig. 4)** (23) and, accordingly, these cells have been shown to be highly sensitive to exogenously-induced DNA damage in healthy state (24,25). However, the potential role of DDR as a contributor of B-cells malfunction has been ill defined in autoimmune conditions.



	Pro-B cell	Pre-B cell	Immature B cell	Mature B cell
Process	*Igh* chain gene assembly	*Igl* chain gene assembly	*Igl* chain gene receptor editing	Immunoglobulin CSR
DSB generation	Multiple RAG-mediated DSBs in all cells	Multiple RAG-mediated DSBs in all cells	RAG-mediated DSBs in some cells	• AID, UNG, APE1 • Multiple AID-induced DSBs in all cells once they are activated
ncDDR	Yes	Yes	Not known	Yes

Figure 4. B cell development requires DDR processes. B cell development proceeds in the bone marrow through distinct steps. From a standpoint of DNA double-strand break (DSB) generation and potential non-canonical DDR signaling, these stages can be generally divided into the pro-B cell stage in which *Igh* gene assembly occurs in all cells, the pre-B cell stage in which *Igl* gene assembly occurs in all cells and the immature B cell stage in which receptor editing occurs in some cells. DNA DSBs are also generated in mature B cells once they are activated and initiate class switch recombination (CSR). AID, activation-induced deaminase; APE1, apurinic/apyrimidinic endonuclease 1; BCR; B cell receptor; RAG, recombination-activating gene; UNG, uracil-DNA glycosylase. From Bednarski et al (23)**.**

DNA damage response (DDR)

Definition and Components

The DNA damage response (DDR) is a mechanism that consists of multiple signal transduction pathways required to meet the challenge of passing down undamaged

DNA to subsequent generations and, thus, maintaining genomic stability (26,27). This response mechanism faces, every day, tens of thousands of damaged DNA lesions per cell (28–30) by activating a complex, dynamic and structured cascade **(Fig. 5)**. In this cascade of events, the DNA sensors molecules (e.g., RPA, Ku, MRN complex) recognize specific genome modifications (base mismatches, single-stranded DNA breaks, DNA adducts, double-stranded DNA breaks) and recruit the following reinforcements: (i) proteins that accumulate at the detected damaged sites and transduce the signal (e.g., ATM, ATR, DNA-PKcs, γH2AX, 53BBP1) and (ii) effector molecules (e.g., CHK2, CHK1, p53, RAD51, BRCA1) that carry out the critical outcome of the cascade (31). These processes, consisting mainly of protein–protein interactions, are usually mediated by post-translational modifications (i.e., phosphorylation, poly(ADP-ribosyl)ation, ubiquitination, SUMOylation, acetylation, and methylation) in order to arrange spatiotemporal protein activity. For instance, ATM, a central regulator of the DNA double-strand breaks (DSBs) response, once recruited and activated, can activate p53 and checkpoint kinase CHK2 through phosphorylation in order to halt the cell cycle at the G1/S checkpoint and extend the time frame for the repair machinery.

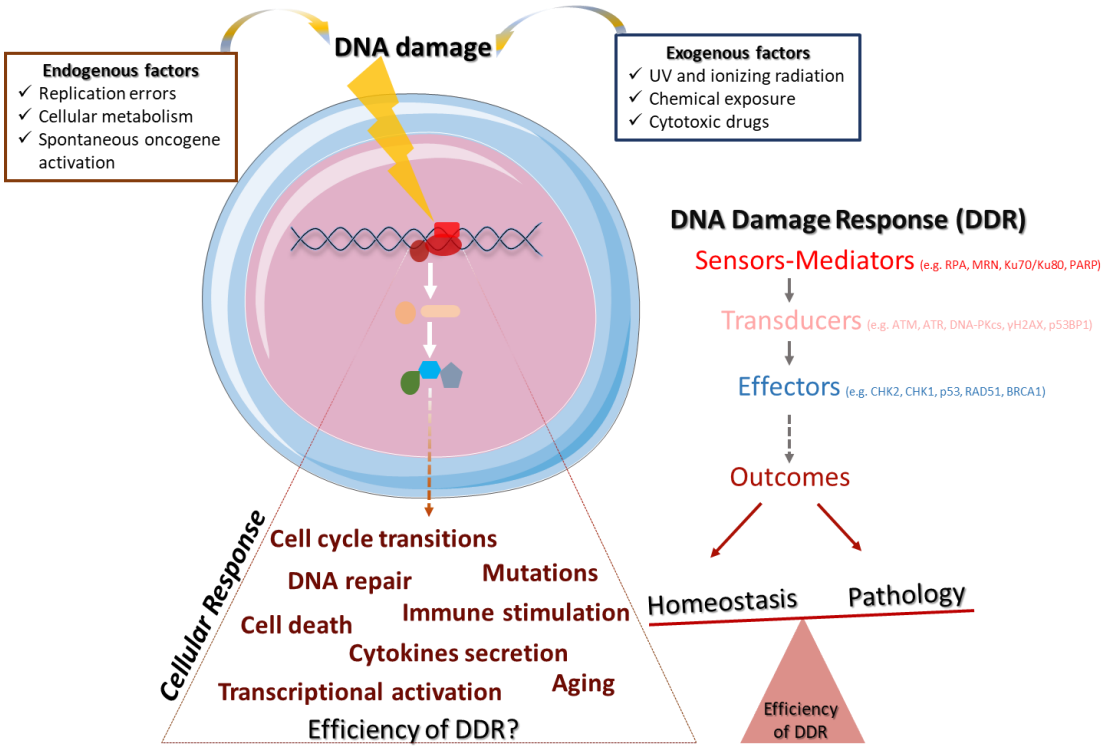


Figure 5. The DDR in health and disease. Endogenous or exogenous agents threaten genome integrity and trigger DDR. DDR requires the stratified activation of involved molecules,

namely, the DNA damage sensors and mediators, the transducers of the signaling and the effectors. Depending on the efficiency of the DDR and the cellular response, the potential outcomes may lead to the restoration of the homeostasis or to pathology. Adopted by Manolakou et al (31).

DNA Repair Pathways

The DNA repair machinery consists of several pathways that usually function throughout the cell cycle, and each of them is responsible to fix a different type of DNA damage. More specifically, there are at least the following five DNA repair pathways that are frequently activated (32): (a) homologous recombination (HR), which repairs DSBs upon the presence of a normal homologous DNA template; (b) non-homologous end-joining (NHEJ), which repairs DSBs without the need for a template; (c) nucleotide excision repair (NER), which repairs bulky DNA lesions and is important for its ability to remove the damage induced by UV; (d) mismatch repair (MMR), which repairs DNA single-strand breaks (SSBs) that occur predominantly during DNA replication and recombination; and (e) base excision repair (BER), which repairs SSBs occurred usually due to oxidation, alkylation and methylation. The potential distinct fates of the DNA damaged cells have been extensively discussed (33–38). While the desired outcome in physiological conditions is the repair of DNA and the restoration of the cell cycle to allow the cells function properly, the outcome may also be prolonged arrest of cell cycle, cell death, tumorigenesis, secretion of inflammatory cytokines (39), and aberrant immune responses (40–43) (**Fig. 5**).

Triggers of DDR

The DDR is triggered upon genome aberrations, which may occur via (a) errors during the physiological context, such as cellular metabolism, e.g., excessive formation of reactive oxygen species (ROS) and oxidative DNA damage (44); (b) DNA replication, e.g., base mismatches (45); and (c) inefficient activity of topoisomerases I and II (26). The DDR operates in conjunction with the immune system to generate immune receptor diversity (such as B cell and T cell receptors) and antibodies during V(D)J recombination, class-switch recombination (CSR) and somatic hypermutation (SHM), where DSBs and/or SSBs in conjunction with the DDR/repair mechanisms are involved in the development of lymphocytes (23,46,47). Notwithstanding, the DDR can also be

activated by DNA-damaging agents including ionizing radiation (IR), ultraviolet light (UV), chemicals and cytotoxic drugs (48). Overall, endogenous and exogenous insults, occurring either randomly or in a scheduled manner, jeopardize the genome integrity and activate the DDR mechanism. Depending on the mechanism's efficiency, it is likely that the damage may result in impaired cellular function.

DDR and the Immune Response

While the DDR comprises critical pathways to control cell function, the immune response comprises specialized cells responsible for mediating the organism's homeostasis. Thus, a defective DDR in immune regulators may lead to deregulated homeostasis, and hence, pathology. A deficient or hyperactive DDR has been extensively documented during tumorigenesis and viral infections, but also in patients with autoimmune diseases and in autoimmune experimental models *in vitro* and *in vivo*, underpinning the role of the DDR in promoting autoimmunity.

Deciphering how the DDR cross-talks with the immune system's functions and affects its responses leading to autoimmunity, remains an open question. While much has been written on the innate components (23,49,50), the DDR involvement in the activation and function of the adaptive immune cells in the non-physiological context remains ill-defined. In general, the following two conditions make the adaptive immune cells prone to the aberrant DDR: (a) physiological processes such as V(D)J recombination, SHM and CSR, and (b) antigen-activation, where adaptive immune cells divide extraordinarily rapidly to ensure an effective immune response, thus jeopardizing the genome integrity due to DNA replication errors. Accordingly, among the blood cell populations, T and B cells have been shown to be highly sensitive to exogenously induced DNA damage (24,25).

DDR and Immunity: A Dubious Relationship That May Culminate in Autoimmunity

The Early Steps and Important Findings

During the development of lymphocytes, the DDR pathways are activated in a well-planned manner, usually either by DSBs (mainly activation of NHEJ) or by abnormal

base pairing (mainly activation of BER and MMR) (23). Although the DDR and immune response mechanisms appear highly coordinated in health, herein, I discuss why and how this balance is disturbed, affecting immune cell responses and subsequently promoting autoimmunity.

Several studies have revealed the presence of DNA damage and the aberrant DDR in either the whole-blood cells or PBMCs (human peripheral blood mononuclear cells) of patients with autoimmune diseases, but only a few have focused on the effects of the mostly implicated cell subsets, being the adaptive immune cells. In particular, autoimmunity occurs when an adaptive immune response is introduced against self-antigens. Under physiological circumstances, adaptive immunity is introduced against foreign antigens (produced by viruses or microorganisms) and is initiated by the activation of antigen-specific T cells. Eventually, it will result in the elimination of the invader through either the T cells (i.e., T cytotoxic) or the formation of antibodies by B cells (i.e., plasma cells), following an interplay with T cells, that will attack the antigens. Nevertheless, in autoimmune responses, there is an abnormal activation of the T and B cells, which leads to the release of autoantibodies against self-antigens, causing tissue damage (51). Therefore, the adaptive immune cells have key roles in the autoimmune response. Consequently, it becomes apparent that in order to delineate the involved pathogenic mechanisms and provide insights into the disease pathogenesis, I need to profile and examine separately the involved cell populations, focusing on their unique properties.

Since the early 1980s, researchers have reported patients with autoimmune diseases presenting with aberrant DDR in their lymphocytes or PBMCs, displaying increased sensitivity to DNA-damaging agents, deficient DNA repair, and oxidative stress (52–55). This phenomenon was considered as a breakthrough in the field of autoimmunity, but the mechanistic insight remains ill-defined to date. During the ensuing years, the need to investigate the effects of the DDR either as causal or causative of an autoimmune disease, and provide a link with immune responses, became more apparent. However, since the immune system deploys complex arrays to function, the direct interplay between the DDR and the immune response becomes extremely arduous to elucidate. Only a few studies have succeeded to provide specific mechanisms that associate autoimmune disease pathogenesis with the DDR, and most

of them do not assign the observed mechanism to a specific cell population. Nevertheless, they have established a strong link between autoimmunity and the DDR, as will be outlined below.

TREX1, a significant component of the DDR, involved in the regulation of DNA repair and in the clearance of cytoplasmic DNA to prevent the activation of innate immunity, has been implicated in autoimmune responses. Respectively, cells deficient in TREX1 appear with ATM-dependent cell cycle arrest, resulting in the defective clearance of DNA in the cytoplasm (56). TREX1 mutations, leading to the loss of its exonuclease activity, have been reported in Aicardi–Goutières syndrome (AGS) and SLE patients (57–59). Although both T and B cells have been shown to contribute to the autoimmune phenotype in Trex1-deficient mice (60), the reasons it promotes the inflammation only to specific organs and not to others, such as the brain and the lungs, which are often affected in SLE and/or AGS, remain unknown. Furthermore, while TREX1 has been shown to participate in systemic autoimmune diseases (i.e., AGS and SLE), its role in organ-specific autoimmunity (i.e., multiple sclerosis, psoriasis, type 1 diabetes, etc.) has yet to be determined.

Another important factor that contributes to the DDR and also influences autoimmunity is p21, which may be activated upon DNA damage via the p53 DDR effector molecule, to inhibit the cell division cycle and DNA replication, and finally promote the repair of the damaged DNA (61,62). Interestingly, p21-deficient mice with a pre-existing mild autoreactive genetic background usually display severe lupus-like autoimmunity glomerulonephritis and promote T cell overactivation (63,64). Notably, the *in vivo* overexpression of p21 directly in T cells restrained the accumulation of effector T subsets (CD4+, CD8+) (62). Exploiting p21-deficient mice models with different autoimmune backgrounds may demonstrate contradictory results (65). Likewise, decreased POLB activity, a crucial enzyme for the repair of damaged DNA, has been linked to SLE. In a pioneering study (66), mice expressing the hypomorphic Polb allele developed an SLE-like phenotype as a result of aberrant V(D)J recombination and a high frequency of SHM. Anti-p53 antibodies that block p53, a crucial DDR effector molecule that regulates DNA repair and cell cycle arrest, and other autoantibodies related to DNA repair components (APEX1, AURKA, POLB, AGO1, HMGB1, IFIT5, MAPKAPK3, PADI4, RGS3, SRP19, UBE2S and VRK1) have been found in

the serum of SLE patients (67,68). Of note, DSBs and deficiencies in DDR molecules, such as ATM, NBS1, MRE11A and also p53, have been observed in rheumatoid arthritis (RA) patients (69). Screening in the serum of patients suffering from autoimmune rheumatic diseases revealed autoantibodies against the DNA repair proteins WRN and MRE11A, as well as against the critical DDR regulators Ku, DNA-dependent protein kinase catalytic subunit and poly(ADP-ribose) polymerase (70). Collectively, these studies have provided sufficient data for the involvement of the DDR in the pathophysiology of autoimmune diseases.

DNA Damage Response in the Adaptive Arm of the Immune System

Linking T Cell DDR with Autoimmunity

T cells and subsets such as helper (CD4+), cytotoxic (CD8+) and regulatory T cells (Tregs) have unique functions that shape the immune response. Dysfunctions in any T subset or the presence of autoreactive T cells have been broadly documented either as causal or causative in autoimmunity. As mentioned above, in developing T-lymphocytes DDR events operate during V(D)J recombination to generate T-cell receptor diversity (TCR) in order to recognize antigens. How the DDR normally regulates this process has been extensively discussed by Bednarski et al. (23). However, how can defects in the DDR be implicated in aberrant T cell-mediated responses in autoimmunity?

To this end, McNally et al. (71) demonstrated that antigen-activated mouse and human T cells in healthy conditions, as well as T cells in the autoimmune disease hemophagocytic lymphohistiocytosis (HLH), exhibit an increased DDR as shown by the elevated levels of classic DDR regulators γH2AX, phospho-p53 (Ser15, Ser46), phospho-ATM (Ser1981), phopsho-CHK2 (T68) and phospho-CHK1 (Ser345). The inhibition of key DDR molecules that regulate the cell cycle, such as CHK1/2 or WEE1, and MDM2, resulted in the selective apoptosis of the pathogenic activated T cells in a HLH murine model, being the CD8+ T cells, and in a multiple sclerosis (MS) murine model (such as EAE), being the CD4+ T cells. This DDR perturbation, termed as PPCA ("p53 potentiation with checkpoint abrogation"), is accomplished though the suppression of the cell cycle checkpoint and the increase in p53 activity, which does not allow the restoration of the damaged cells and leads their apoptosis. The proposed

strategy by the authors does not affect other critical and immunomodulatory T cell subsets, such as naïve, Tregs and resting memory. Therefore, the authors present a potential therapeutic strategy in autoimmunity that targets only the pathogenic activated T cells. In a study published later (72), activated CD4+ T cells restored induced DNA damage compared to resting CD4+ T cells, where the unrepaired damage resulted in cell apoptosis, concluding that DDR/repair is defective in resting CD4+ T cells. The authors also provided evidence that DNA damage sensors (i.e., γH2AX, p53BP1) fail to accumulate at the damaged foci in resting CD4+ T cells, hampering the transduction of the DDR signal towards the repair mechanisms and resulting in apoptosis. Of note, the induced apoptosis relied on JNK/p73 pathway activation (and not on p53 pathway), suggesting an interplay between the DDR and other pathways to shape the cell fate. Resting T cells are non-proliferating, suggesting that the defective DDR is independent of genomic instability due to replication stress. It would be of interest to demonstrate how DDR deficiencies in resting CD4+ T cells affect cell properties with regards to cell differentiation, cytokines secretion and cell communication, and whether they contribute to autoimmune diseases, in order to provide more insights into autoimmune disease pathogenesis and treatment. Moreover, CD4+ and CD8+ T cells displayed increased DSBs in SLE patients when compared with healthy controls and RA (73), as assessed with γH2AX expression levels, which correlated with disease activity. When these cells were subjected to oxidative stress through hydrogen peroxide (H2O2) administration, the accumulated DNA damage was higher in SLE compared to the healthy controls, suggesting defects in the DNA repair mechanisms. Nevertheless, the unanswered questions that arise are as follows: Which is the underlying DDR mechanism that leads to the observed phenotype in SLE T cells? Does this mechanism drive cell behavior in SLE?

The DDR in T cells has also been associated with the cells' DNA methylation patterns in the context of autoimmunity. For example, in SLE, T cells exhibit DNA demethylation, which correlates with T cell autoreactivity. Li et al. (74) showed that the increased expression of growth arrest and DNA damage-induced 45α (GADD45A) gene in CD4+ T cells contributes to autoimmunity in SLE by promoting DNA demethylation of CD11a and CD70, and autoreactivity. The expression levels were proportional to the disease activity. This phenomenon was exaggerated upon UV-

induced DNA damage. The silencing of GADD45A resulted in increased DNA methylation of autoimmune-related genes, following a reduction in T cell autoreactivity. However, in another study (75), T cells were more prone to overactivation in mice lacking the Gadd45a gene. These mice also developed a systemic autoimmune condition resembling SLE. This discrepancy might be due to the congenital Gadd45a deficiency in the mouse model, which affects more cells than T cells, suggesting a more complex than anticipated signaling triggered by the DDR deficiency in various cells that affect the cell responses. Despite this, these articles are in agreement that GADD45A appears to be a key player in autoimmunity. Examining its expression in various cell subsets in large cohorts of autoimmune patients, and further investigating its mechanistic importance in mouse models of both induced and spontaneous autoimmunity, may shed light on the controversial evidence and establish the role of GADD45A in autoimmunity. Moreover, the mechanisms by which GADD45A may affect different organs' homeostasis and whether it is involved in organ-specific autoimmune diseases remain to be elucidated.

Additional research has implicated defective DDR with genomic instability and T cell function in RA. T cells in RA accumulate increased levels of DNA lesions and resist the cell cycle and repair machinery to become either hyperactive or apoptotic. Yang et al. (76) demonstrated that naïve CD4+ T cells in RA fail to activate ATM due to deficiencies in ROS production. This prevents the cells from entering the G2/M critical cell cycle checkpoint that allows the repair of damaged DNA, and thus promotes their differentiation into inflammatory effector cells (Th1 and Th17). The induction of ROS production activated efficiently the ATM pathway and decreased the cells' immunogenicity.

Similar to CD4+ and CD8+ T cells, the DDR mechanisms in Tregs are receiving increased attention since these cells have a pivotal role in the tumor microenvironment where DNA damage usually precedes, and in preventing autoimmunity where the DDR's role is currently emerging. In a recent study from our team (77), the transcriptomic analysis of Tregs in MS, SLE and RA patients revealed elevated expression levels of DDR-related genes as H2AFx, TP53, CHK2 and TP53BP1. The aberrant DDR was confirmed in an experimental mouse model (EAE) of MS by the increased levels of phospho-ATM (Ser1981), p53BP1 and γH2AX proteins. This was attributed to mitochondrial oxidative

stress through the production of mitochondrial (mt) ROS, resulting in cell death, accounting for the decreased numbers of Tregs found in the periphery of the autoimmune patients. Of note, Treg-specific scavenging of mtROS *in vivo* restrained the DDR, reduced apoptosis, and diminished the autoimmune responses. These findings assess Treg functions in autoimmunity with regards to oxidative stress and DDR, and could enable advances in immunotherapy. However, whether the aberrant DDR in Tregs detected in the aforementioned MS, RA and SLE patients is equally important and contributes to all aspects of disease pathogenesis, remains under question. Exploiting autoimmune models for these diseases that allow Treg-specific scavenging of the examined pathways and investigation of the overall disease progress, will strengthen the significance of the results. Also, considering the instrumental role of Tregs not only in autoimmunity, but also in cancer and immune homeostasis, unraveling the involved DDR mechanisms may provide novel insights into the disturbance of immune tolerance mechanisms in health and disease.

Collectively, these studies indicate that T cells exhibit an aberrant DDR in various autoimmune responses. This aberrant DDR may be directly associated with other deregulated processes that are important for cellular homeostasis, such as metabolic processes. The exact circumstances for the observed DDR manifestations and the differential roles of DDR in autoimmunity remain to be defined.

Linking B Cell DDR with Autoimmunity

The DDR events are also essential under physiological settings for the development and the cell-type specific processes of B cells. Even a slight error, during these highly coordinated and programmed processes, may lead to the aberrant DDR compromising the immune responses. Since B cells produce antibodies, and autoantibodies against the DDR/repair molecules have been reported in autoimmune conditions, they are positioned as the obvious suspects for the imbalanced relationship between the DDR and immune response. However, to date, the literature examining the DDR aberrancies in B cells in autoimmunity is limited.

The B cells from systemic autoimmune rheumatic disease (SARD) patients may generate autoantibodies against DDR-related proteins, suggesting that B cells respond to quiescent or lasting DNA damage preceding or during the development of overt

disease. These autoantibodies are directed against Ku, MRE11A, PARP, WRN, p53, PMS1, PMS2, MLH1, and other nuclear proteins that are implicated in the DDR, and their deregulated expression levels have been associated with defective DNA repair (70,78,79). Other autoantibodies, such as 3E10 found in SLE, affect the DDR by binding to DNA and inhibiting DNA repair. It has been proposed that the toxic effect of 3E10 on DNA is achieved when the cell is predisposed in a DNA-damaging environment (80), indicating that cells in autoimmune conditions such as lupus may be already prone to DNA damage by factors unknown so far, and the generation of DNA-damaging antibodies further exacerbates the preexisting deregulated DDR.

Mutations that lead to the decreased expression of the DDR-associated gene POLB, identified in a GWAS, have been associated with SLE (81,82). POLB is a DNA polymerase with a critical role in the BER pathway, therefore constituting an important mediator of the DDR outcome. Researchers generated a mouse model expressing the hypomorphic Polb allele, and highlighted that decreased Polb expression leads to SLE (60). In particular, the mice displayed multiorgan symptoms of SLE, following the altered V(D)J recombination of B cell receptors (whereas no significant differences were detected in T cells) and increased SHM occurring in the later stages of B cell development within the germinal center (GC). Of note, both the B and T (follicular T helper) cells of GCs were increased in this mouse model, with CD4+ T cells being mostly apoptotic. This study provides robust evidence that expression derangement of DDR-associated genes involved mainly in B cell physiology can be associated with autoimmune phenotypes. Although the researchers have focused mostly on B cell properties, and less on T cells, more cells are affected by the decreased Polb expression. Therefore, it would be of interest to investigate additional cell populations affected by Polb deficiency, the effects on cell communication and their contribution to the observed autoimmune phenotype. Furthermore, since POLB appears to be an important regulator of immune responses, it would be intriguing to extend its investigation beyond the systemic autoimmune phenotype and examine the implications for distinct organs' pathologies, therefore extrapolating its role to more autoimmune disorders.

In another recent study (83) with mechanistic insights, the differential expression of DDR-related genes in naïve B cells was sufficient to differentiate a subgroup of

patients with RA with erosive disease from patients with a milder disease. In particular, this group demonstrated low expression of ATM, MRE11A and NBS1 genes of the ATM-related repair cascade of DSBs. Decreased ATM function and activation was associated with a limited BCR repertoire, an increased number of atypical B cells (CD21-/low), and the secretion of pro-osteoclastogenic RANKL and IL-6 cytokines. A loss of ATM expression has also been implicated with defects in the innate immune system enabling bacterial infections (84).

Using B lymphoblastoid cell lines from SLE patients, other studies report limited DNA repair inefficiency with regards to the DSBs repair process (85). In particular, half of the patients exhibited DDR defects, as shown by comet and colony survival assays after DNA damage inducing irradiation, suggesting that the repair mechanisms of damaged DNA are ineffective. Although in this study the authors demonstrate an association of defective DDR with an autoimmune disease, no specific DDR signaling pathway was described. To this end, transcriptomic analysis in several B subsets (rN: resting naïve, T3: transitional 3, aN: activated naïve, SM: isotype-switched memory and DN2: double negative) from SLE patients compared to healthy controls identified deregulated DDR pathways, such as the p53 signaling being positively enriched in all of the SLE B cells and the G2/M cell cycle checkpoint being upregulated in all of the subsets, except for DN2 B cells where it was downregulated (13). DN2 B cells, which is a distinct population of isotype-switched cells, are enriched in SLE patients, and it has been proposed that they may contribute to SLE pathogenesis (86). These data uncover an overall DDR deregulation across the B cell hierarchy and differentiation. Whether this deregulation affects all of the B subsets equally, and how the relationships between the differentially or similarly DDR-affected subsets may exaggerate B cell dysfunctions in SLE, remain to be addressed. Overall, these studies report the aberrant DDR outcomes to be differentially associated with many aspects of the B cell developmental processes and functions in autoimmunity, and they underscore the key involvement of the DDR in the underlying pathophysiology of autoimmune diseases.

In summary, the aberrant DDR beyond the physiological processes of lymphocytes development have been documented in both T and B cells in autoimmunity (**Fig. 6**). Since these cells are essential components of the immune response, these studies put

the DDR forward for further investigation of the pathogenesis of autoimmune diseases.

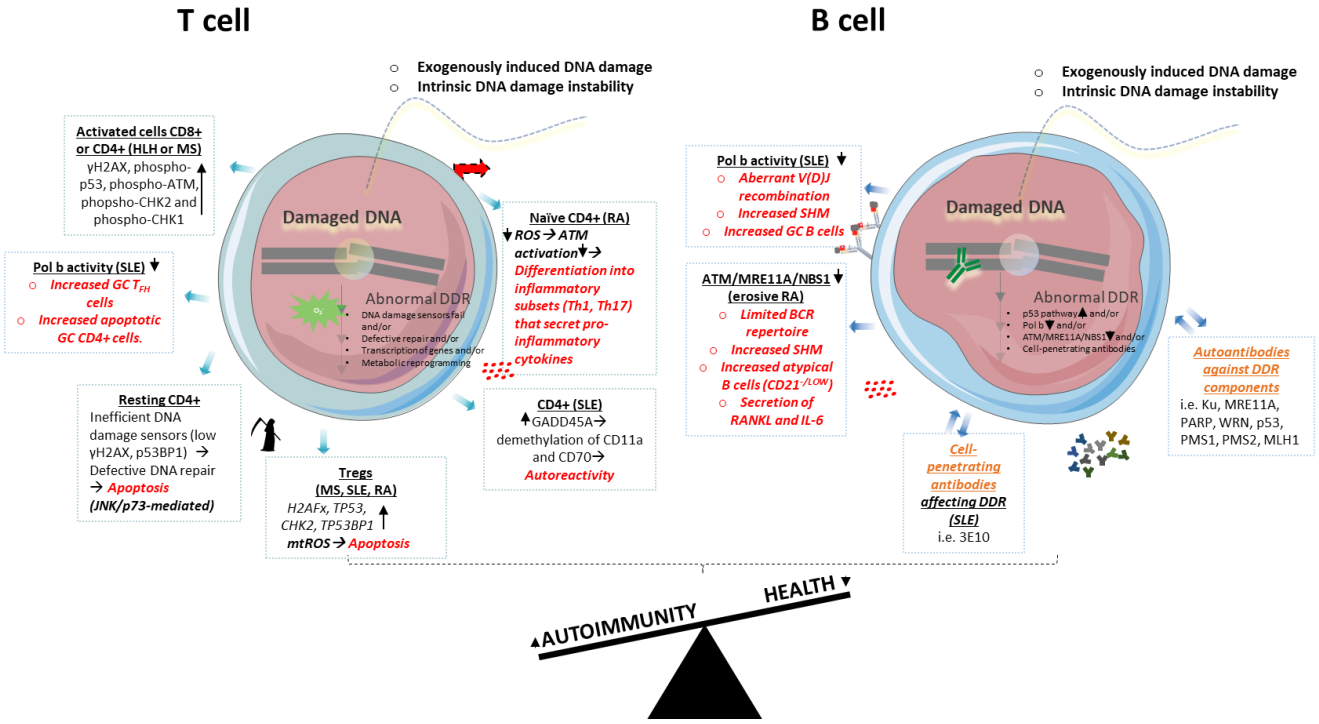


Figure 6. DDR in T and B cells in autoimmune conditions. In autoimmunity, both T and B cells display various defects in DDR molecules, which have been associated with abnormal cellular functions. In autoimmunity, T cells display aberrant expression of DDR genes and proteins, and oxidative stress, which have been associated with alterations in cells' differentiation into immunogenic subsets and/or increased apoptosis (marked with red color). B cells' defects in DDR have been associated with aberrancies in V(D)J recombination, SHM, subsets formation and cytokines secretion (marked with red color). In addition, B cells in autoimmunity produce autoantibodies that may enter nucleus and affect DDR (marked with orange color). DDR: DNA damage response; HLH: hemophagocytic lymphohistiocytosis; MS: multiple sclerosis; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; TFH: T follicular helper; GC: germinal center; ROS: reactive oxygen species; mtROS: mitochondrial reactive oxygen species; Tregs: regulatory T cells; SHM: somatic hypermutation; BCR: B-cell receptor. Adopted by Manolakou et al. (31).

Aberrant DDR in Innate Cells May Exacerbate Aberrant Immune Responses in Adaptive Cells: The Case of Dendritic Cells (DCs)

The generation of an immune response relies on stepwise processes among the involved cell populations. Since the innate immune response precedes the adaptive immune response, it is crucial to discuss, the predominant innate cell component that

primes adaptive immune cell functions, being the antigen-presenting (APC) dendritic cells (DCs). The DCs interact and stimulate antigen-specific T cells, which, accordingly, activate B cells. Consequently, a putative dysfunctional DC, due to aberrancies at its DDR mechanisms, could have an escalating impact throughout the adaptive immune response.

In this direction, researchers have identified the DCs from Nlrp3-/- and Caspase 1-/- mice to exhibit reduced levels of DNA damage and p53-induced apoptosis as a result of effective DNA repair mechanisms, following exposure to DNA-damaging agents such as oxidative H2O2 and genotoxic MSU crystals (87). Of note, high expression levels of Nlrp3 and Caspase 1 have been associated with a plethora of autoimmune diseases (88,89). Therefore, these data imply that the DCs may require decreased Nlrp3 and Caspase 1 expression levels to have an efficient DDR, yet many autoimmune patients are characterized by increased levels and, accordingly, they may undergo a defective DDR. In brief, the inflammation caused by NLRP3/Caspase 1 activation may fuel the DDR in DCs, which in turn exhibit increased immunogenicity. Moreover, the DCs, following the inhibition of the key DDR orchestrator ATM, either genetically or pharmaceutically display delayed maturation, reduced T-cell activation and increased apoptosis, suggesting that ATM is critically involved in their development and functions (90). A loss of ATM function has been implicated in severe autoimmune conditions (83). In this study, ATM function is considered as DNA damage-independent, since DNA damage levels were not reported, suggesting that the DCs might have not been subjected to actual DNA damage. Collectively, these studies document the regulatory function of the DDR or DDR-related molecules on the DCs development; however, they do not provide direct indications for the effects on the subsequent cell interactions within the adaptive immune branch.

The DC-T cells interactions are followed by the synergy between T and B cells, which is essential for the development of appropriate antibodies and the efficiency of adaptive immune response. Defects in their interaction may lead to autoimmune responses (91). Should one of these contributors transmit defective signals to the other due to the aberrant DDR issues, the DDR's defective outcome is propagated through various pathways and cells, and may result in dire consequences in later states, in a cascade-like fashion. For example, in RA patients there is a PD-1hiCXCR5-

Bcl6low T peripheral helper (TPH) cell population that infiltrates inflamed synovia and is involved in the priming of B cell responses (92). Notably, p53 is able to suppress the CHCR5 chemokine receptor through the inhibition of NF-kB activity and BCL6, reinforcing the DDR (93,94). Thus, the DDR in T cells may both affect their responses and determine the B cell response. This may explain why depletion therapies of a single-cell population (i.e., B cell depletion therapies) may not be sufficient for a robust therapeutic response. To that end, further research is required to decipher the DDR-dependent mechanisms of crosstalk among DCs, T and B cells.

The Role of DDR in Other Cells of the Adaptive Immunity: NK Cells, γδ T and NKT Cells

The NK, γδ T and NKT cells represent a bridge between the innate and adaptive responses (95,96) since they are of lymphocytic lineage with innate features. Their involvement in autoimmune diseases has been described both as disease-controlling and disease-promoting (97–99). NK cells have germline-encoded antigen receptors, and therefore they do not undergo V(D)J recombination, whereas γδ T and NKT cells express TCRs derived from V(D)J recombination. All three of the subsets influence T and B cells and their effector actions.

A normal DDR is important for the development of NK cells, and RAG enzymes' functions are involved in DDR outcomes (100). Of note, RAG enzymes (RAG1 and RAG2) have been initially studied for their critical involvement in V(D)J recombination by introducing DNA DSBs (101,102). Notwithstanding, the researchers studied NK cells that do not undergo V(D)J recombination and revealed an additional DDR role for the RAG enzymes with regards to NK cells' expansion, survival and responses. More specifically, RAG deficiency in murine NK cells displayed increased γH2AX levels at steady-state, and was associated with an impaired DDR characterized by DNA-PKcs, Ku80, Chk2 and ATM reduced gene expression. Importantly, RAG deficiencies (RAG1/RAG2) have been associated with autoimmunity (103,104) suggesting that the aforementioned DDR outcomes may be implicated in the pathogenesis of autoimmune diseases.

Another DDR-related molecule that is crucial for NK cells' functions, and also for γδ T, NKT and several T cell subsets, is the NKG2D receptor whose ligands are modulated

by ATM/ATR DDR signaling (105,106). The NKG2D receptor and ligands have been implicated in numerous autoimmune diseases (107–113), since their aberrant expression may lead to the activation of autoreactive effector cells and trigger autoimmune responses. A role of the DDR in the NKG2D receptor and ligands' deregulated functions has not been clearly reported in autoimmune diseases. Yet, human and mouse data in the context of cancer (106) suggest that NKG2D ligand overexpression detected in tumor cells may be due to chronic activation of the DDR in order to trigger the immune system. Finally, Swann et al. (114) reported that NKT cells exhibited a tumor-suppressive role in cancer caused by the p53 deficiency in mice, implying a link of NKT modulatory functions in concert with DDR mechanisms.

Taken together, these studies support the contribution of the DDR to NK, γδ T and NKT cells' immune responses, adding a layer of complexity that involves various immune cell populations being affected by the DDR components. However, a direct causative link of this contribution to the pathophysiology of autoimmune diseases remains to be established.

DDR and Cytokines in Autoimmunity

DDR May Lead to Exaggerated Cytokine Production and Promote Autoimmune Inflammation

Cells produce cytokines that orchestrate all facets of an immune response. In particular, cytokine production allows the communication among cells, and regulates the development and activities of particular cell populations. Pro-inflammatory cytokines contribute to the pathogenesis of autoimmune diseases. Interestingly, the production of pro-inflammatory cytokines, such as IL-6, TNF-α, IFN-γ, has been associated with genome instability following the DDR (115). These cytokines are secreted by a wide range of cell types, including T and B lymphocytes.

In the case of IL-6, Rodier and colleagues (39) demonstrated *in vitro* that the IL-6 response required the persistent activation of the DDR signaling though the DDR proteins ATM, CHK2 and NBS1 (NBS1 expression is usually required for optimal ATM activity). Although, in this study, researchers used mainly fibroblasts, IL-6 is also produced by adaptive immune cells (i.e., B cells) and is essential for the maturation of B cells into antibody-producing cells. In other words, the DDR occurring either in B

cells per se or in other cells that interact with B cells (such as T cells), may increase IL-6 secretion, which stimulates autoantibody production leading to autoimmune disease.

Cell Free DNA may Induce Cytokine Production

Cell-free DNA from apoptotic cancer cells integrates into the genome of neighboring healthy cells to induce cytokine production (such as IL-6, TNF-α and IFN-γ), suggesting that the aberrant DDR and inflammatory response are closely linked pathologies (115,116). In a physiological context, extracellular DNA does not drive genome instability as long as (a) its degradation is fast and (b) it does not integrate into the genome. In general, foreign DNA can integrate into the genome by either exploiting sequence homology-dependent means or by performing illegitimate integration via homology-independent processes (117). However, the involved mechanisms that drive the integration into a specific cell are unclear. Nevertheless, either the integration being homology-dependent or -independent, the involved machineries require adjustments to the cell cycle employing the DDR system. Interestingly, high levels of cell-free DNA have been reported in autoimmune diseases, such as SLE, probably due to defective clearance (118,119). Since, among the immune cell populations, T and B cells display extremely rapid division rates deviating from regular cell cycle checkpoints, they become susceptible to genome instability, facilitating DNA integration into these cells. Therefore, the release of cytokines observed in autoimmune conditions could be associated with the genome instability of T and B cells, caused from the integration of the cell-free DNA into their genome. Thus, the aforementioned aberrancy may follow the defective extracellular DNA clearance seen in autoimmune diseases.

Overall, these studies indicate that the DDR contributes to excessive cytokine release, promoting immune responses towards autoimmunity. Yet, the involvement of particular adaptive immune cells and the DDR signal have not been characterized thus far. Nevertheless, future studies in this field may offer knowledge with tremendous impact on autoimmunity, considering the plethora of cytokines already targeted in the clinic to treat patients with autoimmune diseases (120,121).

Therapeutic Manipulation of DDR in Autoimmunity

Lessons Learnt from Cancer

Genomic instability and DDR deregulation have been extensively investigated in cancer, where they have been linked to tumorigenesis. The development of DDR-targeted drugs in cancer, either genotoxic or against proteins, aims to attenuate the DNA repair ability of the tumor cells in order to facilitate radiotherapy to eliminate the tumor cells, or to promote synthetic sensitivity or lethality (SSL). In particular, synthetic sensitivity diminishes cell divisions, which, when accompanied with additional therapeutic agents such as cytotoxic drugs, may result in cell death. Olaparib (122) is an FDA-approved PARP inhibitor developed in BRCA1/2-defective cells. PARPs are enzymes that sense the DNA damage and contribute to the activation of repair pathways. Their inhibition results in the accumulation of DSBs, which are supposed to be repaired by the HR repair pathway. In cells with BRCA1/2 gene defects, as in the case of ovarian cancers, HR signaling is deficient, and therefore the cells end in cycle arrest and reduced cell viability via synthetic lethality (123).

Pearl at al. (124) have performed an extensive systematic computational analysis to identify direct druggable opportunities in the DDR protein components exploiting large-scale genomic and expression data for 15 cancers, and they discovered possible targets for all of the major DDR pathways. It is important to note that the DDR-based therapeutic strategies do not directly affect the DNA structure and, therefore, they are considered to be nongenotoxic to the patients. Of note, among the characterized current drugs or novel targets, there are the DDR proteins whose deregulation is also implicated with autoimmune diseases, such as CHK1, p53, PARP, MRE11A, ATM.

DDR Targeting in Autoimmune Diseases

In this context, the question that arises is how these targets could be modulated therapeutically in autoimmune diseases. One approach may be to inhibit their expression in pathogenic cells (e.g., antibody-producing B cells in SLE) and test whether this restrains their pathogenic phenotype. Nonetheless, many of these DDR targets have been found downregulated (instead of upregulated as in the case of many cancers) in autoimmune conditions, suggesting that the suppression instead of the upregulation of their expression may be harmful. Yet, this downregulation is

usually observed either in the mRNA or the total protein's levels and does not always mirror the active form (e.g., phosphorylated proteins) of the protein, since most studies have documented numerous phosphorylated DDR-involved proteins overexpressed in autoimmunity. In particular, DDR signaling and outcome is transduced and determined by a series of successive post-translational modifications of the involved molecules. It is likely that there is a negative regulatory loop for the expression of such genes. Targeting the active instead of the inactive DDR proteins could be a reasonable approach.

A DDR-based therapy to suppress the immune response in antigen-activated T cells in the human autoimmune diseases HLH and MS has been proposed by McNally (71). After the screening of DDR-altering small-molecule compounds, they provided evidence *in vivo* and *in vitro* that a combination of therapeutic strategies enhancing the p53 DDR pathway (via targeting MDM2) and attenuating the cell cycle checkpoints (via targeting CHK1 or WEE1) results in the selective elimination of pathological T cells and the treatment of the autoimmune murine models.

In a more forward approach, radiation therapy, which is broadly used for cancer treatment to eliminate cancer cells and enhance the efficiency of other immunotherapies, could be exploited, at lower levels than the one used in cancer, in autoimmunity for the manipulation of the cells' immunogenicity. More specifically, it has been recently shown that radiation therapy may induce TREX1 activity to degrade the accumulated DNA in the pathogenic cells and reduce their immunogenicity (125). Since (a) B and T cells are more sensitive to irradiation; (b) TREX1 activity is decreased in various autoimmune conditions; and (c) B and T cells' immunogenicity is a key contributor to the autoimmunity, researchers could tackle the question whether low-dose irradiation could be exploited to ameliorate autoimmunity.

In light of these developments, I speculate that in the near future selective aspects of autoimmunity may be exploited for anti-tumor therapies. In this direction, the existence of autoantibodies that penetrate into the cell nuclei and threaten the genome integrity and cell viability, has been well documented during the last years (79). For example, autoantibodies, such as 3E10, may have the ability to selectively damage and affect the DDR of the cells that are susceptible to genome aberrations. How these autoantibodies contribute to SLE pathogenesis is not clearly understood. It

is possible that they exaggerate the inflammation and the production of other autoantibodies following the signaling perturbation they provoke into the cell by binding to DNA. It is therefore expected that SLE patients have an increased risk of certain types of cancer (126–129). Accordingly, this type of cell-penetrating autoantibody that damages DNA may have a therapeutic potential against cancer (130). Specifically, since these autoantibodies do not appear to affect healthy cells, but only those that are predisposed to DDR defects, they could be exploited for (a) the selective elimination of tumor cells and (b) the treatment of immunogenic cells in the context of autoimmunity that demonstrate DDR defects, as in the case of adaptive cell subsets, by transporting molecules able to restore the cellular properties (131).

In summary, targeting the DDR aberrations in autoimmunity could be accomplished within the following frames: (a) targeting the DDR molecules with abnormal expression in specific cell populations, where DDR plays a role beyond the physiological cellular functions resulting in the formation of immunogenic cells; and (b) since the DDR per se is critical for the cell survival and expansion, targeting the DDR in immunogenic cell populations—even if the DDR has not been associated with the cell's unfavorable functions—in order to eliminate the pathogenic cells. Since the adaptive immunity is a fundamental driver of autoimmunity, targeting selective adaptive immune cell types could result in improved therapeutic outcomes in the treatment of autoimmune diseases. The use of cell-penetrating antibodies that may transport therapeutic agents into the damaged cells, may also hold a therapeutic potential in autoimmunity. In this direction, there is an emerging need for developing suitable animal models that reliably reflect the pathogenesis of autoimmune diseases and allow the preclinical experimentation of cell-based therapeutic strategies.

Multiomics: Transcriptomics and Proteomics

The protein-coding genes are transcribed to RNA, further processed to mRNA and finally translated into proteins which usually require additional modifications (i.e., post-translational) to become fully functionable. Interestingly, even the non-protein coding genes, which account for the majority of the genome, generate RNA although not translated into protein (132). The central dogma of biology viewed RNA as a molecular intermediate between DNA and proteins, which are considered the primary

functional read-out of DNA. However, omics studies in the past decade have shown that while only ~3% of the genome encodes proteins, up to 80% of the genome is transcribed (133).

The total of RNA molecules, coding and noncoding, constitutes the transcriptome while the complete set of proteins expressed by an organism constitutes the proteome. The proteome is an expression of an organism's genome. The transcriptome is examined with transcriptomics, a set of high-throughput genomic methods giving information on sequence and abundance of transcripts. The proteome is investigated with proteomics, a set of techniques developed to approach a high-throughput level providing identification of peptide abundance, modification, and interaction. The analysis and quantification of proteins has been revolutionized by MS-based methods and, recently, these have been adapted for high-throughput analyses of thousands of proteins in cells or body fluids. Moreover, transcriptomic studies (RNA-Seq) identify thousands of novel isoforms and showing a larger than previously appreciated complexity of the protein-coding transcriptome (134). Transcriptomics and proteomics provide unique information and supplement each other, while changes in transcript levels may not necessarily correspond to similar changes in protein levels (135). Omics technologies have begun to unveil the complex molecular networks involved in human pathologies towards understanding development and disease. In this direction, Panousis et al. (136) have made an exceptional contribution to the field by performing whole-blood RNA-seq analysis in 142 individuals with SLE and 58 healthy controls and identifying a disease-susceptibility signature that may facilitate personalized care.

AIM OF THE STUDY

SLE patients demonstrate increased levels of DNA damage, defective DNA repair and polymorphisms in genes required for maintaining genomic stability. Effective DNA damage response is crucial for the generation of antibodies by B cells while excessive production of autoantibodies is a universal feature of the disease. However, the contribution of DNA damage response (DDR) signaling in pathogenic cell responses involved in SLE remains elusive.

Herein, by the use of transcriptomic, proteomic, microscopy, flow cytometry and *ex vivo* studies, I sought to characterize the DDR profile in peripheral cell populations, focusing on the most pathogenic population of SLE, B cells. More specifically, the main goals of this study are: a) to identify the DDR pathway involved in B cell autoreactive responses in SLE; b) to decipher how this pathway may be affected by the pathogenic SLE landscape and, finally; c) to investigate whether specific targeting of DDR components may alleviate the pathogenic phenotype of B cells representing a potential therapeutic target in SLE.

Noting how critical DDR deregulation may be for SLE pathogenesis, in parallel experiments disease-involved tissues, such as the skin, were analyzed with regards to DDR to understand the molecular events underlying tissue injury and response.

MATERIALS AND METHODS

Human Subjects

Peripheral blood samples were obtained from patients with SLE (n=51, classified by the 1997 American College of Rheumatology criteria (137)) and healthy controls (HC, n=103). Skin biopsies were obtained from 2 HC and 3 SLE individuals. At the time of sampling, all patients had moderate to high disease activity (SLEDAI ≥ 8,) and, in the vast majority, had not received cytotoxic drugs 6 months prior to donation. All patients and healthy individuals were recruited through the Rheumatology and Clinical Immunology Unit, 4th Department of Internal Medicine, “Attikon” University Hospital and the Department of Rheumatology, "Asklepieion" General Hospital (both in Athens, Greece). Informed consent was obtained from all individuals prior to sample collection (Athens, Greece, protocol 10/22-6-2017). All patients omitted any treatment dose for at least 24h prior to blood drawing. To exclude a non-specific effect of the inflammatory milieu, I used peripheral blood samples from patients with ankylosing spondylitis (AS) (n=4) as additional controls, because they show evidence of broad inflammatory response, but without pathogenic B cell responses and production of autoantibodies. **Tables 1, 2, 3 and 4 include the demographics of all the human samples used.**

Animal Studies

All procedures in mice were in accordance with institutional guidelines and were reviewed and approved by the Greek Federal Veterinary Office (1044/1319) (Athens, Greece). New Zealand black-female x New Zealand white-male F1 mice (i.e., NZB/W-F1) spontaneously develop an autoimmune syndrome resembling human SLE (138). NZB (NZB/OlaHsd) and NZW (NZW/OlaHsd) mice were purchased from Envigo. NZB/W-F1 were considered diseased when exhibiting ≥100 ng/dL of urine protein (following 6 months of life), and pre-diseased at 10 weeks old. The animals were maintained in the Biomedical Research Foundation Academy of Athens (BRFAA) animal facility. All NZB/W-F1 mice used in the experiments were female. Mice were housed 6 per cage in a temperature- (21-23^{o}C) and humidity- controlled colony room, maintained on a 12h light/dark cycle (07:00 to 19:00 light on), with standard food

(4RF21, Mucedola Srl, Italy) and water provided ad libitum. All mice in the animal facility were screened regularly by a health-monitoring program, in accordance to the Federation of European Laboratory Animal Science Association (FELASA), and were free of pathogens (139).

Proteomics

Sample preparation

B cells were isolated simultaneously (to avoid batch effects) from frozen peripheral blood mononuclear cells (PBMCs) of SLE patients and HC (n=11/condition) with EasySep™ Human B Cell Isolation Kit (Cat. #17954, STEMCELL) following DNase treatment according to the guidelines of EasySep. The purified cell population was subjected to complete cell lysis using a buffer consisting of 4% SDS, 100 mm Tris/HCl, 100 mm DTT, pH 7.6 and incubated at 95 °C for 5 min. The lysed samples were further sonicated for 30 min in a water bath. The protein extracts were purified from debris by centrifugation for 20 minutes at 17Kxg. The supernatants were transferred to clean tubes and processed according to the Single-Pot Solid-Phase-enhanced Sample Preparation (SP3) method of Hughes (140), without acidification and including a step of protein alkylation in 100 mM Iodoacetamide. Digestion was carried out for continuous shaking at 1400 rpm at 37 °C using 0.25 µg Trypsin/LysC mixture in a 25 mM ammonium bicarbonate buffer. Next day, the magnetic beads were removed and the peptidic samples were further purified by Sp3 peptide cleanup and evaporated to dryness in a vacuum centrifuge. The dried samples were solubilized in Buffer A, sonicated for 5 minutes and the peptide concentration was determined by measuring the absorbance at 280 nm using a nanodrop.

Ultrahigh pressure nanoLC

Each sample was analyzed three times *(technical replicates)*. Approximately 0.5 µg peptides were pre-concentrated with a flow of 10 µL/min for 4 min using a C18 trap column (Acclaim PepMap100, 100 µm x 2 cm, Thermo Scientific) and then loaded onto a 50 cm long C18 column (75 µm ID, particle size 2 µm, 100Å, Acclaim PepMap100 RSLC, Thermo Scientific). The binary pumps of the HPLC (RSLCnano, Thermo Scientific)

consisted of Solution A (2% (v/v) ACN in 0.1% (v/v) formic acid) and Solution B (80% (v/v) ACN in 0.1% (v/v) formic acid). The peptides were separated using a linear gradient starting with 5% B up to 27.5% B in 58 min stepped to 40% B in 2 min and finally reaching 99%B and remaining there for 5 min and then allowed to equilibrate for 20 minutes with a flow rate of 300 nL/min. The column was placed in an oven operating at 50°C.

Tandem mass spectrometry (*MS/MS)*

The eluted peptides were ionized by a nanospray source and detected by an Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) operating in a data dependent mode (DDA). The peptides were measured from 350-1500 m/z, using a resolving power of 120K for MS1, AGC at 3e6, maximum injection time of 100ms, followed by 12 MS/MS of the most abundant 2^{+}-4^{+} charged ions using a resolving power of 15K, AGC at $1e^{5}$, maximum injection time of 22ms, and an isolation window of 1.2 m/z at 28 NCE and a dynamic exclusion of 30s. The software Xcalibur (Thermo Fisher Scientific) was used to control the system and acquire the raw files and internal calibration was activated using a lock mass of m/z 445.12003.

Data Analysis

The raw files were searched, using the Proteome Discoverer 2.4, against the *Homo sapiens* reference proteome FASTA database downloaded from Uniprot on 19/09/19 (containing 95959 protein sequences) and the ProteomeTools_HCD28_PD spectral library using the multiple peptide search (MPS) option activated and using serially the MSPepSearch and SequestHT nodes. The protein dynamic modifications assessed were oxidation +15.995 Da (M), deamidation +0.984 Da (N, Q) and the N-terminal variable modifications of acetylation +42.011 Da, Met-loss -131.040 Da (M) and Met-loss+Acetyl -89.030 Da (M). Carbamidomethyl / +57.021 Da (C) was set as a static modification. The result filtered for high confident peptides, with enhanced peptide and protein annotations. Only master proteins were evaluated. The quantified abundances were based on intensity values and were normalized to the total peptide amount. The statistical evaluation between the Control group and SLE Patient protein normalized abundances was performed using the Proteome Discoverer software

(pairwise background-based t-test). The minimum percentage of replicate features was set to 60 %. 4995 proteins identified with at least two peptides.

Enrichment analysis of proteomic data

A list of 1,094 differentially expressed proteins based on FDR<0.05 and at least 2 peptides expressed were used as input for the enrichment analysis using STRING(141) and QIAGEN IPA (142) (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA) tools. FDR<0.05 was used to determine significance for enriched GO and KEGG terms in STRING analysis and FDR<0.2 to determine significance for enriched Canonical Pathways in IPA analysis.

RNA-Seq

Published data in fastq format were downloaded and processed as reported below (13,136). Quality of sequencing was assessed using FastQC software (143). Raw reads in fastq format were collected, trimmed for adapter content and low quality bases (Q<30) at the 3' end using cutadapt(144) and aligned to the human genome (hg38 version) using STAR 2.6 algorithm(145). Gene quantification was performed using HTSeq(146). Gencode annotation file version 29 was used for the annotation. Differential expression analysis was performed using edgeR package in R (147). For the whole blood human dataset, age and gender were used as covariates.

Enrichment analysis of RNA-Seq data: Gene Set Enrichment Analysis (GSEA)(148) was performed in order to reveal enriched signatures in our gene sets based on the Molecular Signatures Database (MSigDB) v.7.0. Gene sets were ranked by taking the –log10 transform of the p-value multiplied by the FC. Significantly upregulated genes were at the top and significantly downregulated genes were at the bottom of the ranked list. GSEA pre-ranked analysis was then performed using the default settings. Enrichment was considered significant by the GSEA software for FDR (q-value) <25%.

Human Cell Isolation from Peripheral Blood

Heparinized blood (10ml) was collected from healthy subjects and individuals with SLE. PBMCs were isolated on Histopaque-1077 (Sigma) density gradient. Briefly, blood was diluted 1:1 with PBS and layered over Histopaque medium. Tubes were

centrifuged at 500 g for 30 min with no brake at room temperature. PBMCs layer was collected and cells were washed with PBS. In the cases neutrophils were needed in addition to PBMCs, cells isolation was done on double gradient of Histopaque-1077/Histopaque-1119 (Sigma). In brief, blood was diluted 1:2 with PBS and layered over Histopaque-1077/Histopaque-1119 (1:1). Tubes were centrifuged at 1200 x g for 30 min with no brake at room temperature. Neutrophils were collected at the interface of the Histopaque 1119 and Histopaque 1077 layers and washed with PBS. For the isolation of total B cells, EasySep™ Human B Cell Isolation Kit (Cat. #17954, STEMCELL) was also used in some experiments (for the proteomics and the cultures of cells) following Histopaque-1077 protocol.

Flow Cytometry and Cell Sorting

For analysis and isolation of immune cells, single-cell suspensions from fresh human PBMCs or neutrophils were stained with conjugated antibodies: CD19, CD4, CD8, CD25, HLA-DR, CD14, CD16, CD66b **(Table 5)**. All sorted cells were 7-AAD negative (Cat. 420404, Biolegend). For the intracellular staining **(Table 5)** (Foxp3, γH2AX, Ki67, pATM, cPARP1) cells were fixed and stained using Foxp3 Staining Set (Cat. 00-5523-00, eBioscience) according to manufacturer's instructions. Cells were sorted on a FACS ARIA III (BD Biosciences) using the BD FACSDIVA v8.0.1 software (BD Biosciences). For analysis of cultured cells following EasySep™ Human B Cell Isolation, cells were stained with conjugated antibodies: CD19, CD40, CD80, BAFF, HLA-DR, IgD, CD27, CD38, IgM, IgG and for Ki67 **(Table 5)** intracellular staining as aforementioned. For apoptosis detection upon administration of DDR pharmaceutical inhibitors, cultured B cells were further stained with CD19 and then with Annexin V (Cat. 640908, Biolegend) with 7-AAD were used according to manufacturer's instructions. Analysis was performed with FlowJo software.

For analysis of peripheral B cells from mice, PBMCs were isolated from blood on Histopaque-1077 (Sigma) density gradient. Then, PBMCs were stained with conjugated antibody against B220/CD45R and γH2AX (ser139). Foxp3 intracellular staining kit was used for the staining of γH2AX. Data acquisition was performed on a FACS ARIA III (BD Biosciences) and the BD FACSDIVA v8.0.1 software (BD Biosciences). Analysis was performed with FlowJo software.

Cell cycle assessment

For the cell cycle analysis via flow cytometry 200,000-500,000 B cells/sample (following EasySep™ Human B Cell Isolation from PBMCs) were first stained extracellularly with anti-CD19 antibody in 200 μl 5% FBS/PBS buffer for 10 min, RT, to ensure B cell purity and then, following washing with PBS cells were fixed and stained for Ki67 and γH2AX using Foxp3 Staining Set according to manufacturer's instructions. At the end, cells were stained with 5 μl/sample 7-AAD cellular DNA content marker in 200 μl 5% FBS/PBS buffer for 15 min, RT, washed with PBS, and then resuspended in 300 μl 5% FBS/PBS. Cells were analyzed at BD FACSCelesta™ using the BD FACSDIVA v8.0.1 software. Linear scale was used for 7-AAD. This method allows the assessment of γH2AX expression across apoptosis, G0, G1, S and G2/M phases. For the analysis of B cells upon ATRi, B were cultured for 3 days with IFNα (850 U/ml), IL-21 (50 ng/ml) / CpGb (2.5 μg/ml) / sCD40L (1 μg/ml) survival and mild proliferation stimuli in the presence or absence of ATRi (2 μM) or DMSO (control) in P96 round wells (200,000 cells/well), and then stained and analyzed as mentioned above.

For the cell cycle analysis via confocal microscopy, B cells were cultured for 45h under the following conditions: a) SLE B cells with IL-21/ CpGb/ sCD40L cocktail for survival and mild induction of proliferation, as described above, b) HC B cells treated with IFNα and IL-21/ CpGb/ sCD40L cocktail (SLE-like B cells) and c) HC B cells with IL-21/ CpGb/ sCD40L cocktail (control condition). After 45h of culture, cells were exposed to additional 3h of culture with EdU (5 μM) to capture cells being in S-phase of the cell cycle and then, following EdU wash-off, I proceeded with the Click-iT™ EdU Cell Proliferation protocol for Imaging (Cat. C10337, Thermo Fisher Scientific) along with simultaneous staining of pH3 (1:100, for detection of G2/M mitotic cells), pATR (T1989) (1:100) and Hoechst 33342 (nuclear counterstain). At least 80 cells per human subject were analyzed. The secondary antibodies were Alexa fluor 555 anti-mouse IgG (1/500) for pH3 and Alexa fluor 647 anti-rabbit IgG (1/200) for pATR **(Table 5)**.

Preparation of skin biopsies

Skin biopsies were formalin-fixed and paraffin-embedded as previously described (149) and were cut using a microtome into 5 μm thick sections on slides.

Immunofluorescence

For the cell immunofluorescence experiments, cells were seeded in coverslips pretreated with poly-L lysine, fixed with 4% PFA for 15 min at room temperature (RT) and washed twice with PBS. Cells were blocked and permeabilized with 1% BSA dissolved in PBS containing 0.1% Triton X-100 (blocking buffer) for 30 min at RT. Next, cell seeded slides incubated with primary antibodies and DAPI in blocking buffer at RT for 1h, followed by three washes with PBS containing 0.1% Triton X-100 and then by secondary antibodies for 45 min at RT in dark. Finally, cells were mounted with ProLong™ Diamond Antifade Mountant (Cat. P36961, Thermo Fisher Scientific) and visualized using inverted confocal live cell imaging system Leica SP5. Puncta/cell or intensity/cell were calculated using a macro developed in Fiji software (150). The primary antibodies in the immunofluorescence were against γH2AX (Ser139) (1/200), pATR (Thr1989) (1/100), pChk1 (Ser345) (1/50), p-p53 (S15) (1/100), pChk2 (T68) (1.2/100), p95/NBS1 (1/100), cleaved Caspase 3 (Asp175) (1/800), pDNA PKcs (S2056) (1:150) and the secondary antibodies were Alexa fluor 555 anti-mouse IgG (1/500), Alexa fluor 488 anti-rabbit IgG (1/500), CF® 555 anti-rabbit IgG (1/1000) **(Table 5)**. For the analyses, 60-100 cells per human subject (corresponding to a minimum of 4 different fields of the coverslip) were observed for each marker under confocal microscopy.

For the skin biopsies, sections were first deparaffinized and rehydrated as following: 2x15 minutes in xylene, 4 minutes in 100% ethanol, 4 minutes in 95% ethanol, 4 minutes in 70% ethanol, 4 minutes in 50% ethanol, 4 minutes in distilled water. Sections were blocked and permeabilized with 5% BSA dissolved in PBS containing 0.2% Triton X-100 (blocking buffer) for 1h at RT. Next, sections were incubated with primary antibodies and DAPI in blocking buffer at RT for 1h, followed by three washes with PBS and then by secondary antibodies (diluted in blocking buffer) for 1h at RT in dark. Sections were mounted and visualized as previously described.

Cell culture and chemical inhibition

Healthy B cells were cultured following isolation from fresh PBMCs from healthy individuals (EasySep™ Human B Cell Isolation Kit) in a 37^{o}C humified incubator with 5% CO_2 while plated in 96-Well Round-Bottom plate (Sarstedt) in a concetration of 1.5x10^5 cells/well. Cells were cultured in RPMI 1640 medium with GlutaMAX™ (Cat. 61870036, Gibco) supplemented with 10% (vol/vol) heat-inactivated FBS (Cat. 10270106, Gibco), pen-strep (100 U/ml and 100 µg/ml, respectively; Cat. 15140m Gibco), sodium pyruvate (1 mM; Cat. 11360070, Gibco), HEPES (10 mM; Cat. 15630106, Gibco) and 2-Mercaptoethanol (0.05 mM; Cat. 31350010, Gibco). Also, all cultured B cells, irrespective of experiment, were supplemented with a "survival/mild proliferation" cocktail of IL-21 (50 ng/ml; Cat. 200-21, Peprotech), CpG-B (2.5 µg/ml; Cat. HC4039, HycultBiotech) and sCD40L/CD154 (1 µg/ml; Cat. 11343345, ImmunoTools). To mimic DDR in SLE B cells, IFNα (Cat. 11200-1; pbl assay science) was added to cells at 850 U/ml. For chemical ATR inhibition, Berzosertib (i.e., ATRi) (Cat. S7102, Selleckchem) was added to cells at 2 or 5 µM and for Chk1 inhibition, CHIR-124 (i.e., Chk1i) (Cat. HY-13263, Selleckchem) was added to cells at 50 or 100nM (details on legends).

Measurement of immunoglobulins and cytokines

Released immunoglobulins were measured with LEGENDplex™ Human Immunoglobulin Isotyping Panel (8-plex) according to the manufacturer's instructions following the collection supernatants at day 7 from cultured B cells treated with IFNα (850 U/ml) with or without ATRi (2 µM). Released cytokines were measured with LEGENDplex™ Human B Cell Panel (13-plex) following the collection supernatants at day 2 from cultured B cells treated with IFNα (850 U/ml) with or without ATRi (5 µM). Data acquisition was done on a FACS ARIA III (BD Biosciences) and the BD FACSDIVA v8.0.1 software (BD Biosciences). Analysis was performed with LEGENDplex™ Data Analysis Software.

IRF1 knockdown assay

For IRF1 gene knockdown, Silencer® Select siRNA (i.e., siIRF1) was used (100 nM; Cat. 4392420, Ambion) and Silencer® Select Negative Control siRNA (i.e., siNeg) was used

as a control (100 nM; Cat. 4390843, Ambion). Cells were transfected with the siRNAs with Lipofectamine 2000 (Cat.11668019, Invitrogen™) according to the manufacturer's instruction. Briefly, $3x10^5$ cells were plated per well in 96-Well Flat-Bottom plate in 100 µl/well Opti-MEM™ I Reduced Serum Medium (Cat. 31985070, Gibco) and the appropriate siRNA with the transfection reagents were added for 5h. After 5h, the supernatant was discarded and medium as well as "survival/mild proliferation" cocktail were added. After 42h hours of the initial plating, IFNα was added to both wells with siIRF1 and siNeg. For gene expression analysis, RNA was isolated 2 days after the initial plating, for protein expression analysis, cells were collected 4 days after the initial plating and for cell subsets and proliferation assays 5 days after the initial plating.

Chromatin Immunoprecipitation experiment (ChIP)

To analyse the molecular interactions of ATR in B cells upon IFNα stimulation, ChIP experiment was carried out. In brief, $4x10^5$ cells were plated per well in 96-Well Round-Bottom plate in medium, "survival/mild proliferation" cocktail and IFNα. Cells were collected 6h after the culture with the aforementioned reagents and ChIP was performed with Magna ChIP™ A/G Chromatin Immunoprecipitation Kit (Cat. 17-10085, Merck) according to the manufacturer's protocol. The antibodies used were against IRF1 (Cat. 8478, Cell Signaling) and control IgG (Cat. 3900, Cell Signaling). Detection and analysis of ChIP precipitates were performed by real-time qPCR using primers for the promoter region of ATR. In all cases, data (Ct values) derived from the input sample were used for normalization by the "per cent of Input (% IP)" method and presented as fold of change relative to control anti-IgG IPs. The sequences of the core consensus response element for IRF1 were identified on ATR promoter sequence using Gene Transcription Regulation Database (GTRD) (151) and Eucaryotic promoter database (EPD) (152). The primers sequences are presented in the following table.

Primer name	Orientation	Sequence
ATR loc1	F	TGCTGTAATCTTGTGAGGTAGACA
	R	GGGATTGGGAGTTACAGGCC
ATR loc2	F	TCTTGCTTCTCTGTGCCTCC
	R	GGCTTCTTTCTCAGCACCGA
ATR loc3	F	CACTAGTCAACCACGCCAAC
	R	CCCGGGTCCTATGCAGAAAA

Quantitative PCR Analysis (real-time RT-qPCR)

Cells were lysed in Buffer RA1 (Macherey-Nagel) and RNA was extracted using a NucleoSpin RNA XS isolation kit according to the manufacturer's instructions. First-strand cDNA synthesis was performed using PrimeScript™ RT-PCR Kit (Cat. RR037A, Takara). QPCR was carried out using the Kapa Sybr Fast Universal kit (Cat. KK4602, Kapa Biosystems). Relative expression of target genes was calculated by comparing them to the expression of the housekeeping genes ACTINB or GAPDH.

Primers that were used for real-time RT-qPCR are presented in the following table

Gene	Orientation	Sequence
ATR	forward	GGAGATTTCCTGAGCATGTTCGG
	reverse	GGCTTCTTTACTCCAGACCAATC
ATM	forward	TGTTCCAGGACACGAAGGGAGA
	reverse	CAGGGTTCTCAGCACTATGGGA
IRF1	forward	CCAAGAGGAAGTCATGTG
	reverse	TAGCCTGGAACTGTGTAG
ACTINB	forward	CTCTTCCAGCCTTCCTTCCT

	reverse	AGCACTGTGTTGGCGTACAG
GAPDH	forward	GCACCACCAACTGCTTAG
	reverse	GCCATCCACAGTCTTCTG

Western blotting

Whole cell extracts were lysed with vortex (vortex every 5 min for 20 min with 1 min breaks in between, in ice) in RIPA mix (150 mM sodium chloride, 50 mM Tris-HCl (pH 8.0), 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease (1X, Cat. 1183617001, Roche) and phosphatase (1:100, Cat. P5726, Sigma) inhibitor, then they were centrifuged full-speed for 10 min at 4°C and the supernatant was collected (protein lysate). Protein amounts were determined using DC protein assay (Cat. 5000112, Bio-Rad) according to manufacturer's instruction. Beta-mercaptoethanol (6X) was add to the samples and they were heated for 5 min at 95°C. Cell extracts were resolved using 4–20% precast polyacrylamide gel (Cat. 4561094, Biorad), and transferred to nitrocellulose membrane using a transfer apparatus according to the manufacturer's instructions (BioRad). The protein loading amount per well was determined to 20 μg. Membranes were saturated with 5% non-fat milk diluted in TBS-0.1% Tween 20 (TBS-T) for 1h and incubated with primary antibodies overnight at 4°C and with anti-mouse-HRP or anti-rabbit-HRP secondary antibodies for 1h. Blots were developed with enhanced ECL (Cat. 34580, Thermo Scientific™) according to the manufacturer's instructions. For the analysis, quantification of phospho-protein expression has been performed by utilizing the normalization with the corresponding total protein: both phosphorylated and total proteins were first normalized with housekeeping protein expression. Antibodies are depicted in **Table 5.**

Quantification and statistical analysis

Statistical analysis was performed taking into account the experimental setup using paired or unpaired Student's t-test, one-way or two-way ANOVA in GraphPad Prism v8.0.1 software, as indicated in the figure legends. Data are presented as means ± S.E.M. P value <0.05 was considered as indicative of statistical significance. All P values and n are reported in the figure legends. The investigators were not blinded to the

identities of the samples. Compared samples were collected and analyzed under the same conditions. Each experiment was repeated at least three times. For legendplex experiments, it was one run per assay comprising of 3 independent experiments where samples were collected.

RESULTS

Enriched DDR in peripheral blood cells of SLE patients

To initially investigate whether there is aberrant DDR in the peripheral blood cell populations in SLE, GSEA analysis was performed in whole blood cells of SLE patients with various treatments or severity status compared to age-sex matched HC exploiting the RNA-seq dataset of Panousis et al. (136). The analysis revealed a positive enrichment of DDR- related pathways such as the DNA repair and the G1/S and G2/M DNA damage checkpoints in all SLE patients **(Fig. 7).**

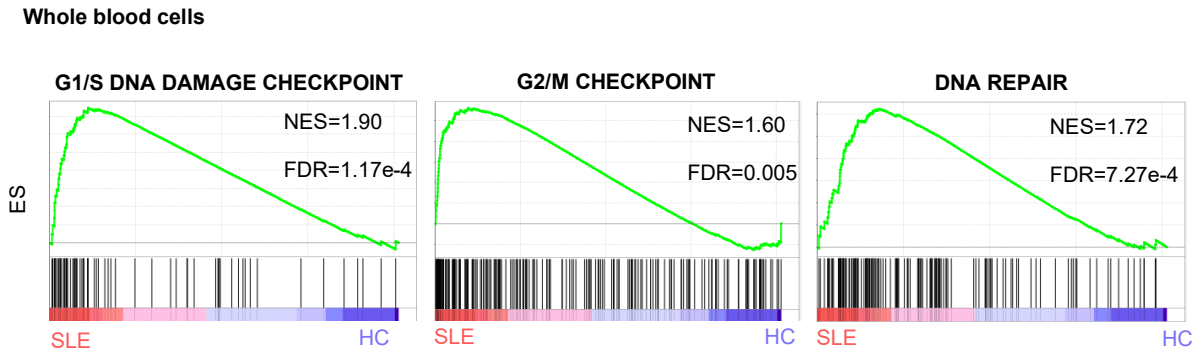


Figure 7. DNA damage response in whole blood cells of SLE patients. GSEA analysis plots (FDR<0.25) depicting aberrant expression profile in DDR-related gene sets of whole blood cells in SLE patients (n=142) compared to HC (n=58) utilizing a publicly available RNA-seq dataset (136). ES: enrichment score, NES: normalized enrichment score.

In order to delineate the observed phenotype, peripheral blood was collected from active SLE patients (SLEDAI ≥ 8) and age/sex-matched HC, and the expression of γH2AX, a classic indicator of global DDR activation, was evaluated in distinct immune cell populations involved in SLE pathogenesis such as B ($CD19^+$), T helper ($CD4^+$), T cytotoxic ($CD8^+$), T regulatory ($CD4^+CD25^+Foxp3^+$), classical monocytes ($HLA\text{-}DR^+CD14^+CD16^-$), intermediated monocytes ($HLA\text{-}DR^+CD14^+CD16^+$), non-classical monocytes ($HLA\text{-}DR^+CD14^-CD16^+$) and neutrophils ($CD66b^+$) cells via analyses of flow cytometry and/or confocal microscopy following cell sorting **(Fig. 8A, B, Fig.9)**. Interestingly, among the cell populations analyzed, B cells demonstrated the most prominent increase of γH2AX expression in SLE patients compared to HC **(Fig. 9)**. The

patients recruited for this study had never received major immunosuppressive therapy to exclude the possibility that the DDR was triggered by drugs.

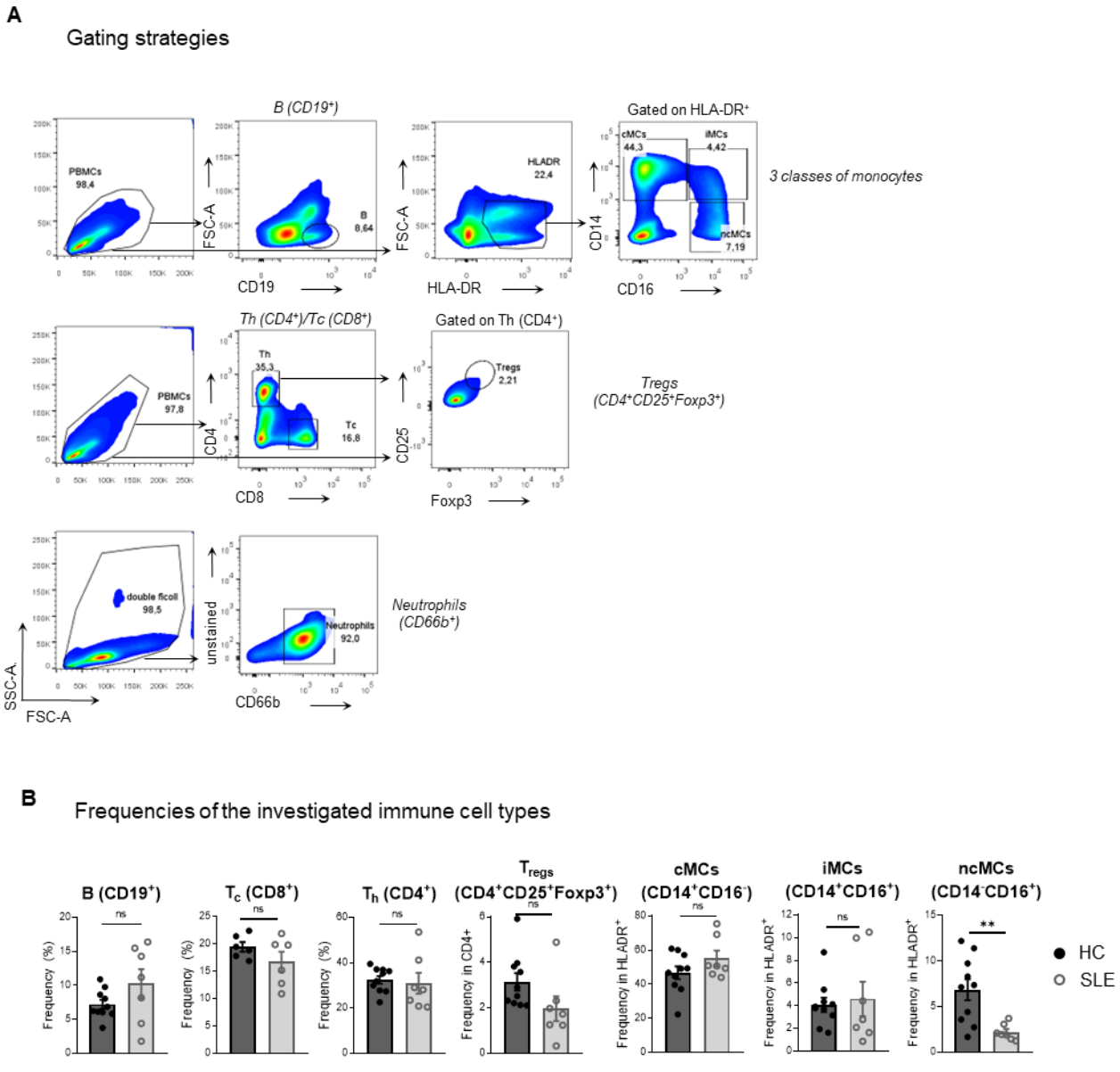


Figure 8. Representative flow cytometry gating strategy for identification of immune cells. **(A)** Gating strategies for the flow cytometry-sorted (for confocal) and/or flow cytometry-analyzed immune cells, following PBMCs or neutrophils isolation from the peripheral blood (see methods). **(B)** Immune cell frequencies of the samples used (SLE; n=7 or 6, HC; n=10 or 6) (unpaired t-test).

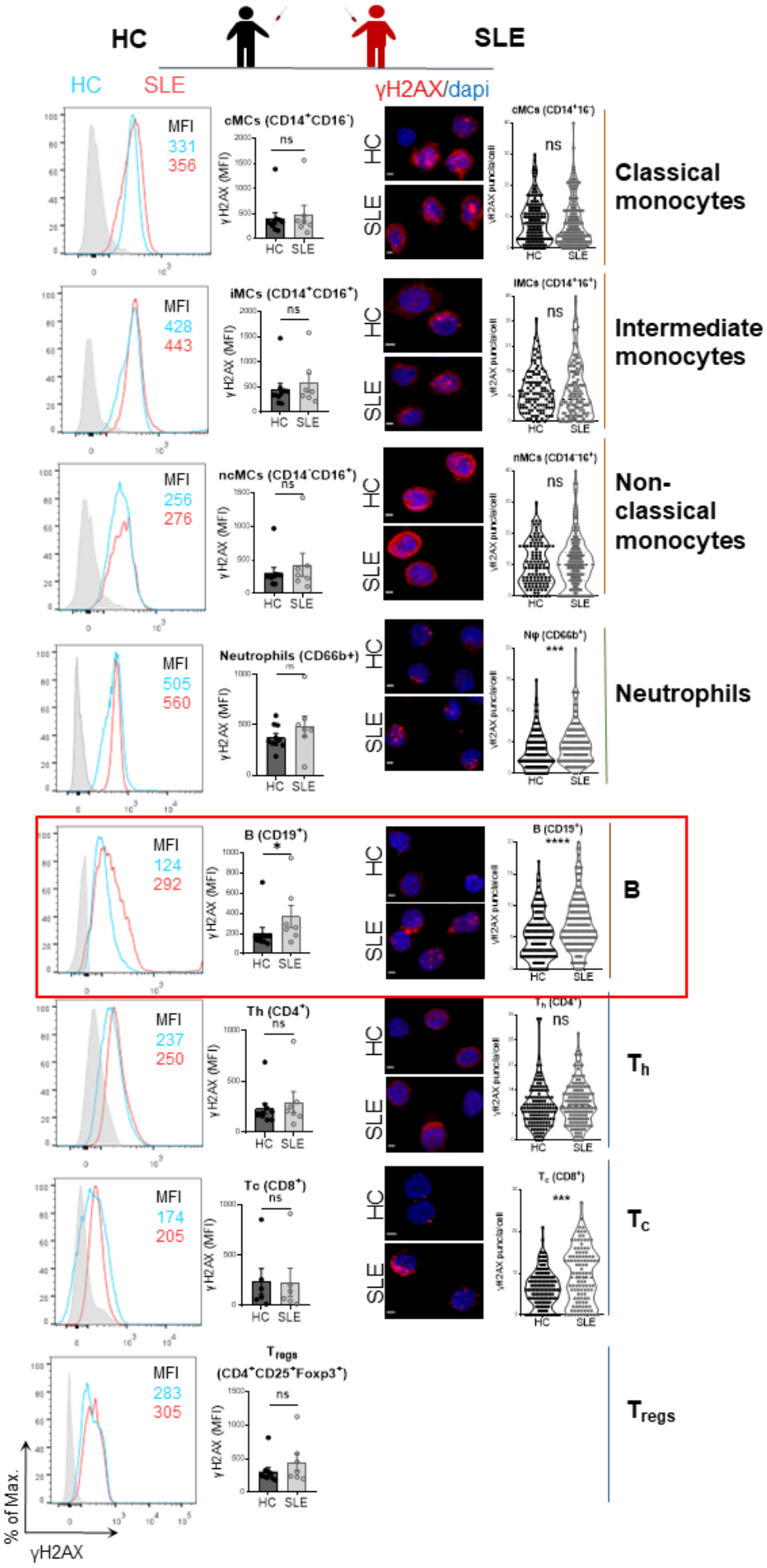

HC
SLE
HC
SLE
γH2AX/dapi
MFI
331
356
cMCs (CD14+CD16-)
ns
γ H2AX (MFI)
HC SLE
HC
SLE
cMCs (CD14+16-)
ns
γH2AX puncta/cell
HC SLE
Classical monocytes
MFI
428
443
iMCs (CD14+CD16+)
ns
γ H2AX (MFI)
HC SLE
HC
SLE
IMCs (CD14+16+)
ns
γH2AX puncta/cell
HC SLE
Intermediate monocytes
MFI
256
276
ncMCs (CD14-CD16+)
ns
γ H2AX (MFI)
HC SLE
HC
SLE
nMCs (CD14-16+)
ns
γH2AX puncta/cell
HC SLE
Non-classical monocytes
MFI
505
560
Neutrophils (CD66b+)
γ H2AX (MFI)
HC SLE
HC
SLE
Nφ (CD66b+)
***
γH2AX puncta/cell
HC SLE
Neutrophils
MFI
124
292
B (CD19+)
*
γ H2AX (MFI)
HC SLE
HC
SLE
B (CD19+)
****
γH2AX puncta/cell
HC SLE
B
MFI
237
250
Th (CD4+)
ns
γ H2AX (MFI)
HC SLE
HC
SLE
Th (CD4+)
ns
γH2AX puncta/cell
HC SLE
Th
MFI
174
205
Tc (CD8+)
ns
γ H2AX (MFI)
HC SLE
HC
SLE
Tc (CD8+)
***
γH2AX puncta/cell
HC SLE
Tc
MFI
283
305
Tregs
(CD4+CD25+Foxp3+)
ns
γ H2AX (MFI)
HC SLE
Tregs
% of Max.
γH2AX

Figure 9. DNA damage investigation in peripheral immune cells in SLE highlights B cells. Screening of peripheral blood immune cells of SLE compared to healthy controls (HC) for DNA damage detection: Expression of γH2AX DNA damage detection marker in B (CD19+), T helper (CD4+), T cytotoxic (CD8+), T regulatory (CD4+CD25+Foxp3+), classical monocytes (HLADR+CD14+CD16-), intermediated monocytes (HLA-DR+CD14+CD16+), non-classical monocytes (HLA-DR+CD14-CD16+) and neutrophils (CD66b+) cells of SLE patients compared to HC via flow cytometry (SLE; n=7 or 6, HC; n=10 or 6) and/or confocal microscopy (FACS-sorted cells; SLE: n=2-3, HC: n=3). Representative histograms showing overlay of unstained cells (grey), stained SLE cells (red) and stained HC cells (light blue), and immunofluorescence confocal microscopy images. Cells on confocal are labeled with DAPI nuclear staining (blue). Scale bar: 2μM. Results are expressed as mean ± SEM. Unpaired Student's t test was applied, $p \geq 0.05$ (ns), * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ****$p<0.0001$. ES: enrichment score, HC: healthy controls, Th: T helper, Tc: T cytotoxic, Tregs: Tregulatory, cMCs: classical monocytes, iMCs: intermediate monocytes, ncMCs: non-classical monocytes, Nϕ: neutrophils, MFI: mean fluorescent intensity.

In support of these findings, increased expression levels of γH2AX DDR marker were also identified in peripheral blood B cells of NZB/W F1 diseased compared to pre-diseased lupus murine model **(Fig. 10A, B)**. In addition to the peripheral blood, among the rest examined tissues (i.e., spleen, renal lymph nodes and bone marrow) in murine lupus, elevated γH2AX expression was also observed in the bone marrow (BM)-derived B cells ($CD19^+$) indicating that deregulated DDR is present since B cell development.

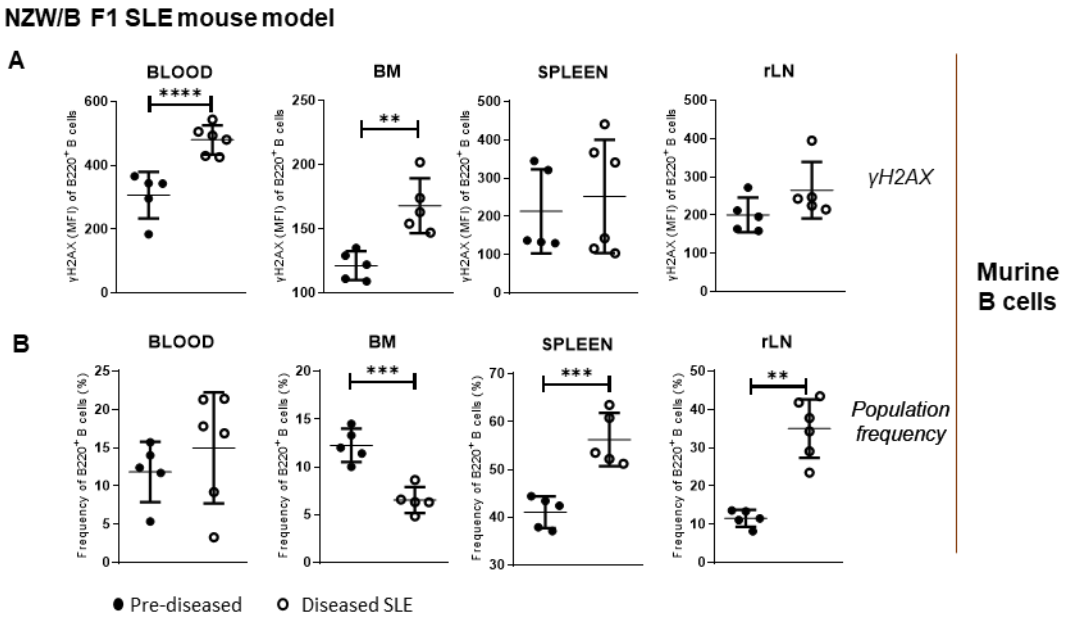


Figure 10. Increased DDR in murine SLE B cells. (A) Expression of γH2AX DNA damage detection marker in total B ($B220^+$) cells of the peripheral blood, the bone marrow (BM), the spleen and the renal lymph nodes (rLN) of NZB/W F1 SLE diseased mouse model (n=5) compared to pre-diseased (n=6) through flow cytometry. **(B)** B cell frequencies across the different tissues**.** MFI: mean fluorescent intensity. Unpaired t-test, ** p<0.01, *** p<0.001, **** p<0.0001.

Aberrant DDR is feature of SLE B cells

To identify DDR mechanisms with functional importance of relevance to a B cell pathogenic role in SLE, I then assessed the proteomic profile of total B cells isolated from the peripheral blood of patients with active SLE (SLEDAI ≥ 8) which had never received major immunosuppressive therapy and age/sex-matched HC (**Fig. 11A, Tables 1, 2 with demographics)**. Principal component analysis (PCA) of total proteins

clearly exhibited a strong separation between the subjects of the two conditions **(Fig. 11B)**. IPA pathway analyses revealed key known features involved in SLE pathology, such as complement activation, cell activation, cytokine production (IL-1, IL-4, IL-6, IL-10), antigen presentation, BCR activation, NF-kB activation and mTOR signaling being deregulated in SLE B cells compared to HC **(Fig. 11C)**. Interestingly, B cells from SLE subjects exhibited enriched DDR mechanisms. In particular, DNA repair, G2/M, G1/S, CHK proteins and other DDR-related pathways were overrepresented in the proteomic signature of SLE B cells compared to HC **(Fig. 11C)**. In support of these findings, γH2AX expression levels were found again elevated in B cells of a separate small cohort of SLE patients compared to HC **(Fig. 11D)**.

Since DDR comprises cell-cycle checkpoint control, I sought to examine whether elevated γH2AX expression in SLE B cells is associated with a specific phase of the cell-cycle. Thus, cell cycle analysis was performed via flow cytometry upon simultaneous assessment of Ki67 proliferation specific marker, 7-AAD cellular DNA content marker and γH2AX in freshly isolated B cells from SLE patients and HC. Despite the fact that γH2AX was overexpressed in the SLE compared to HC B cells, no statistically significant difference was noted in any cell phase, suggesting that the SLE-associated upregulation of γH2AX may be independent of cell-cycle phase **(Fig. 11E)**.

Finally, to investigate whether we can extrapolate our proteomics data on the overall deregulated DDR to the B cells transcriptome and, also, to explore whether a specific B cell subpopulation prevails in the DDR signature, GSEA analysis of B cell subsets from patients with SLE and HC was performed exploiting a publicly available RNA-seq dataset (13). The results indicated that almost all major SLE B cell subpopulations are involved in the altered DDR including resting naïve, transitional 3, isotype-switched memory, double negative 2 and antibody-secreting B cells **(Fig. 11F).** Taken together, these data indicate that B cells exhibit a profound DDR in individuals with SLE.

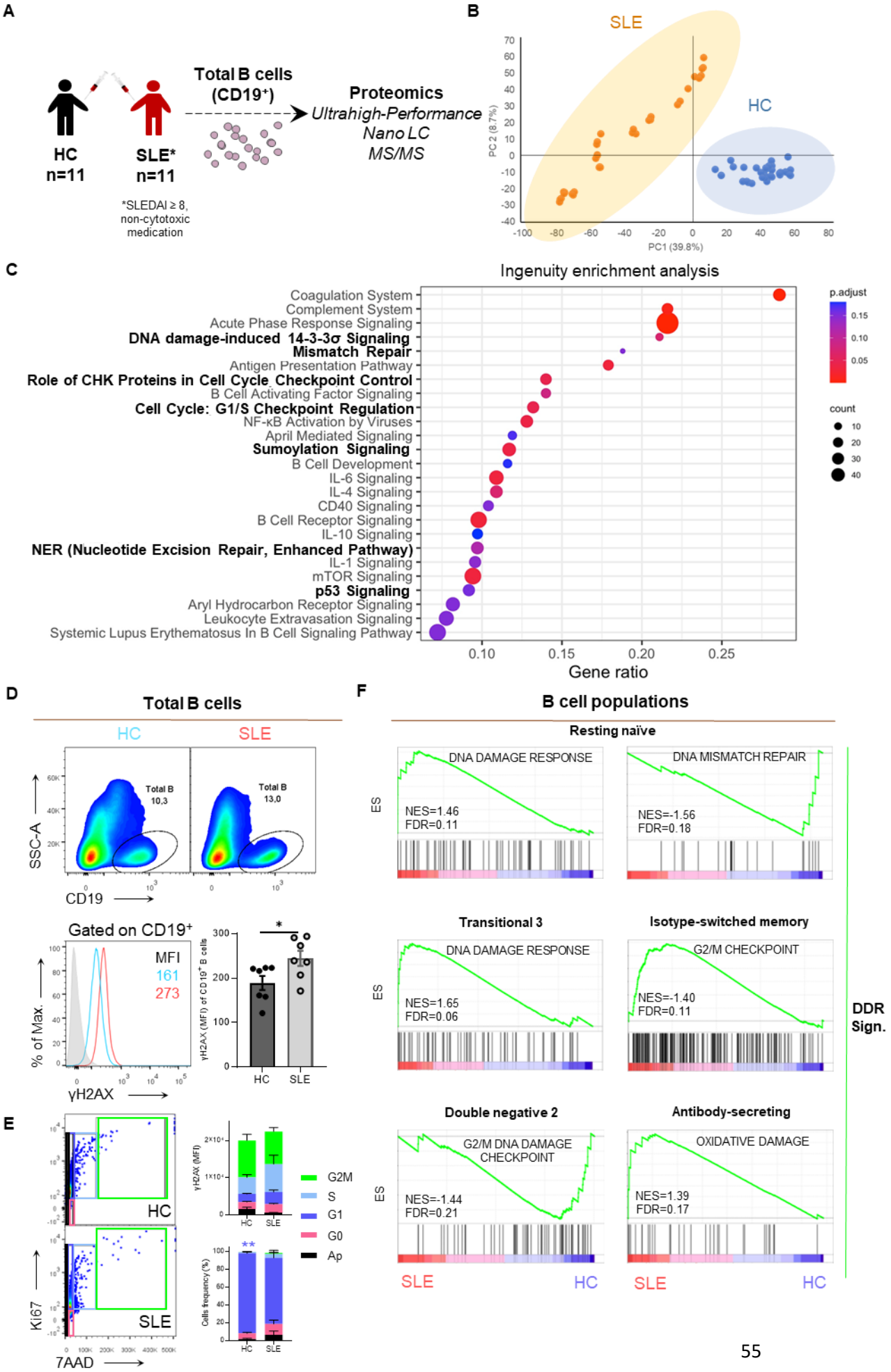

A
HC
n=11
SLE*
n=11
*SLEDAI ≥ 8,
non-cytotoxic
medication
Total B cells
(CD19+)
Proteomics
Ultrahigh-Performance
Nano LC
MS/MS
B
SLE
HC
PC 2 (8.7%)
PC1 (39.8%)
C
Ingenuity enrichment analysis
Coagulation System
Complement System
Acute Phase Response Signaling
DNA damage-induced 14-3-3σ Signaling
Mismatch Repair
Antigen Presentation Pathway
Role of CHK Proteins in Cell Cycle Checkpoint Control
B Cell Activating Factor Signaling
Cell Cycle: G1/S Checkpoint Regulation
NF-κB Activation by Viruses
April Mediated Signaling
Sumoylation Signaling
B Cell Development
IL-6 Signaling
IL-4 Signaling
CD40 Signaling
B Cell Receptor Signaling
IL-10 Signaling
NER (Nucleotide Excision Repair, Enhanced Pathway)
IL-1 Signaling
mTOR Signaling
p53 Signaling
Aryl Hydrocarbon Receptor Signaling
Leukocyte Extravasation Signaling
Systemic Lupus Erythematosus In B Cell Signaling Pathway
Gene ratio
p.adjust
count
D
Total B cells
HC
SLE
SSC-A
CD19
Total B
10,3
Total B
13,0
Gated on CD19+
MFI
161
273
% of Max.
γH2AX
γH2AX (MFI) of CD19+ B cells
E
HC
SLE
Ki67
7AAD
G2M
S
G1
G0
Ap
Cells frequency (%)
F
B cell populations
Resting naïve
DNA DAMAGE RESPONSE
NES=1.46
FDR=0.11
DNA MISMATCH REPAIR
NES=-1.56
FDR=0.18
Transitional 3
DNA DAMAGE RESPONSE
NES=1.65
FDR=0.06
Isotype-switched memory
G2/M CHECKPOINT
NES=-1.40
FDR=0.11
Double negative 2
G2/M DNA DAMAGE CHECKPOINT
NES=-1.44
FDR=0.21
Antibody-secreting
OXIDATIVE DAMAGE
NES=1.39
FDR=0.17
ES
SLE
HC
DDR Sign.

Figure 11. Proteomic and transcriptomic analyses identify DDR as a feature of SLE B cells. **(A)** B cells were isolated using magnetic bead-based approach from the peripheral blood of SLE patients and healthy controls (HC) (n=11 individuals/group) for proteomic analysis. **(B)** Principal component analysis (PCA) using the expression of all proteins for SLE patients and HC in technical triplicates. **(C)** Ingenuity enrichment analysis (IPA) using the 1094 differentially expressed proteins (FDR<0.05) between SLE and HC. Selected enriched canonical pathways (FDR<0.2) are shown. DDR-related pathways are indicated in bold. **(D)** Flow cytometric analysis of PBMCs gated on CD19+ for assessing DDR activation in B cells of SLE patients and HC (n=7 individuals/group) with γH2AX. Representative plots of frequencies and histogram showing overlay of unstained cells (grey), stained SLE (red) and HC (blue) cells are depicted. Statistical significance was obtained by unpaired Student's t test, *p<0.05. **(E)** Flow cytometric analysis for assessing γH2AX expression across cell-cycle phases in magnetic-bead isolated B cells of SLE patients and HC (n=6 individuals/group). Cell-cycle analysis was performed using Ki67 and 7-AAD. The comparison involves the same phase of the two groups (SLE and HC) (two-way ANOVA). **(F)** GSEA plots for DDR alterations across B cells subsets (resting naïve, transitional 3, isotype-switched memory, double negative 2, antibody-secreting) of SLE patients (n=9) compared to HC (n=12) utilizing a published RNA-seq dataset (13), ES: enrichment score, NES: normalized enrichment score, FDR<0.251046 cut-off. MFI: mean fluorescent intensity. Results are presented as mean ±SEM.

Activation of ATR pathway drives DDR in SLE B cells

Next, I sought to characterize the specific molecular mechanism underlying the DDR by examining master kinase proteins and DDR components, namely ATR, ATM, DNA-PKcs, Chk1, Chk2, p53 172 and p95/NBS1, in SLE and HC B cells **(Fig. 12A)**. Because the activation and transduction of the DDR signaling usually requires protein phosphorylation, most targets were investigated in their phosphorylated form (153,154). Increased levels of pATR (T1989), pChk1 (Ser345), p-p53 (S15) and p95/NBS1 were found in SLE compared to HC B cells, but no differences in pATM (Ser1981), pChk2 (T68) 176 and pDNA-PKcs (S2056) levels via immunofluorescence microscopy or flow cytometry **(Fig. 12B**). The activation of the ATR pathway in SLE B cells was also confirmed by western blot analysis in separate individuals **(Fig. 12C)**. These data indicate that the ATR/Chk1 pathway is mainly activated in SLE B cells, while ATM/Chk2 and DNA-PKcs are not critically involved.

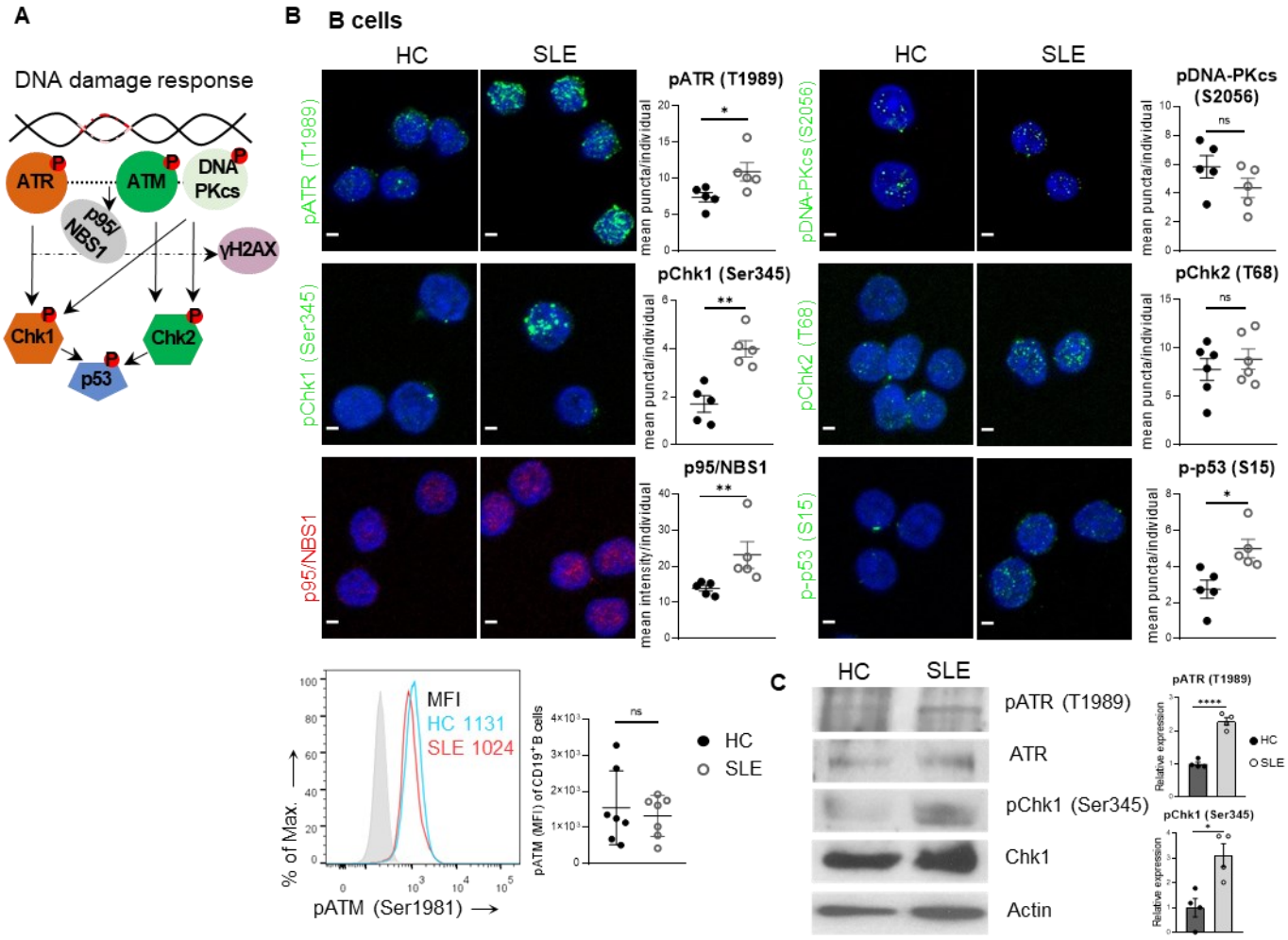


Figure 12. ATR/Chk1 DDR pathway is activated in SLE B cells. (A) Schematic representation of the main DDR pathways being the ATR/Chk1, ATM/Chk2 and DNA-PKcs. **(B)** Investigation of DDR contributors and immunofluorescence confocal microscopy images or representative histogram of stained SLE healthy controls (HC) B cells (CD19+, isolated by FACS-sorting or magnetic-beads). Representative confocal images for pATR (Thr1989) (green) (n=5 individuals/group), pChk1 (Ser345) (green) (n=5 individuals/group), p95/NBS1 (red) (n=5 individuals/group), pATM (Ser1981) (n=7 individuals/group), pDNA-PKcs (S2056) (green) (n=5 individuals/group), pChk2 (T68) (green) (n=6 individuals/group) and p-p53 (S15) (green) (n=5 individuals/group). Analyzed results for pATR, pChk1, pDNA-PKcs, pChk2 and p-p53 are depicted as mean puncta/cell per individual, for pATM as MFI per individual and for p95/NBS1 as mean intensity/cell per individual. For pATM, representative histogram showing overlay of unstained cells (grey), stained SLE (red) and stained HC cells (blue) is depicted Scale bar: 2μM. **(C)** B cells were isolated from SLE patients and HC (n=4 per group) using magnetic bead-based approach and were used for western blot analysis with pATR (T1989), ATR, pChk1 (Ser345) and Chk1 antibodies. Actin blotting was used to confirm equal loading of each sample. Quantification of pATR and pChk1 expression has been performed by utilizing the

normalization with the total ATR and Chk1 protein, respectively. Both phosphorylated and total proteins were normalized with actin expression. Representative samples depicted. Results are presented as mean ± SEM. *p<0.05, **** p<0.0001 (unpaired Student's t test).

To provide additional evidence that ATR-mediated DDR pathway is specifically associated with SLE pathogenic features and not secondary to the inflammatory milieu of the disease, I examined pATR protein levels in B cells isolated from the periphery of patients with ankylosing spondylitis (AS) **(Table 3 with demographics)**. AS is an autoinflammatory disease, wherein, unlike SLE, B cells do not have a major role in pathogenesis and type I IFN signature is absent (155). Nonsignificant changes were noted in pATR protein levels in AS B cells when compared to HC **(Fig. 13A, B)**.

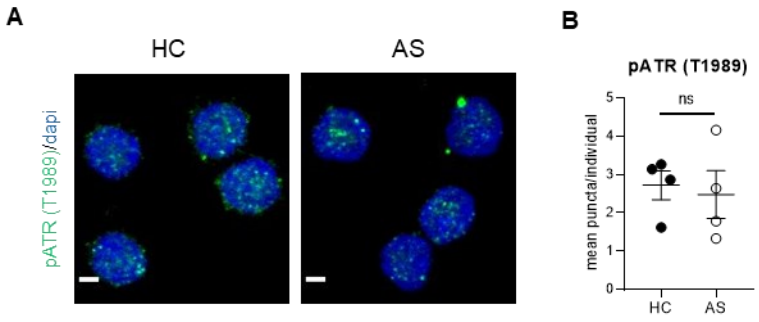


Figure 13. ATR is not activated in AS B cells. **(A)** Representative images of B cells from individuals with ankylosing spondylitis (AS) or HC (n=4 per condition) stained with anti-pATR (Thr1989) antibody and then with a fluorochrome-conjugated secondary antibody (green), were captured by confocal microscopy. Nuclei were stained with DAPI (blue). Scale bar: 2µM. **(B)** Images of B cells analyzed for pATR staining as mean puncta per individual (unpaired t-test).

DDR machinery may induce apoptosis and/or proliferation and, in particular, ATR/Chk1 activation is anticipated to affect cell proliferation (156,157). Yet, neither the proliferation (Ki67) nor the apoptosis (cleaved PARP1 and cleaved caspase 3) differed in SLE B cells when compared to HC, indicating that ATR/Chk1 pathway deregulation may not have a significant impact on these processes in SLE and that other cell responses may be affected **(Fig. 14)**. Although proliferation rate did not differ significantly between SLE and HC B cells **(Fig. 14)**, the statistically significant

decrease of G1-phase derived B cells **(Fig. 11E)** along with the proteomic and transcriptomic data indicating deregulated G1/S and G2/M checkpoints **(Fig. 11C, F)** in SLE compared to HC**,** prompted us to further investigate ATR activity in proliferative (S phase) and mitotic (G2/M phase) B cells**.** To this end, I examined pATR expression levels in S- (EdU^+) and G2/M- ($pH3^+$) phase derived cells, following *ex vivo* activation (IL-21/ CpGb/ sCD40L cocktail for survival and mild induction of proliferation) (158–164) and EdU exposure of peripheral total B cells from SLE patients and HC **(Fig. 15A)**. The results indicated that at both SLE and HC the ATR pathway was upregulated in EdU^+ compared to EdU^- B cells, while $pH3^+$ cells exhibited increased pATR expression compared to $pH3^-$ cells only in lupus environment. Nonetheless, the pATR expression in EdU^+ SLE B cells was significantly higher compared to EdU^+ HC B cells, while it did not present a significant change in $pH3^+$ SLE B cells when compared to $pH3^-$ SLE B cells. These data suggest that although eventually overall proliferation rate is not altered, ATR may have a more active role in the growth and division of B cells in SLE compared to HC **(Fig. 15B, C)**.

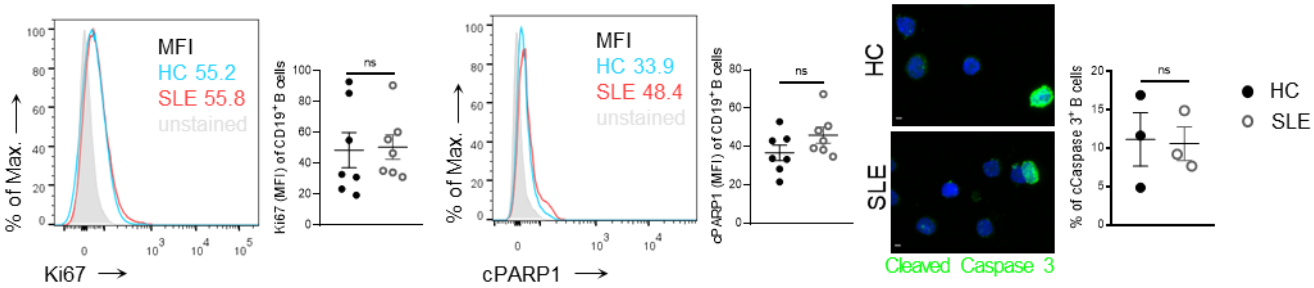


Figure 14. ATR/Chk1 DDR activation in SLE B cells does not correlate with proliferation nor apoptosis. Assessment of proliferation (Ki67) and apoptosis (cleaved PARP1 and cleaved caspase 3) in SLE patients compared to HC via flow cytometry (n=7 individuals per condition) and/or confocal microscopy (n=3 individuals per condition). For cleaved caspase 3 staining, positive signal is depicted with green and cells are quantified as positive or negative utilizing DAPI nuclear staining (blue) (n=3 individuals per condition). Scale bar: 2μM. MFI: mean fluorescent intensity.

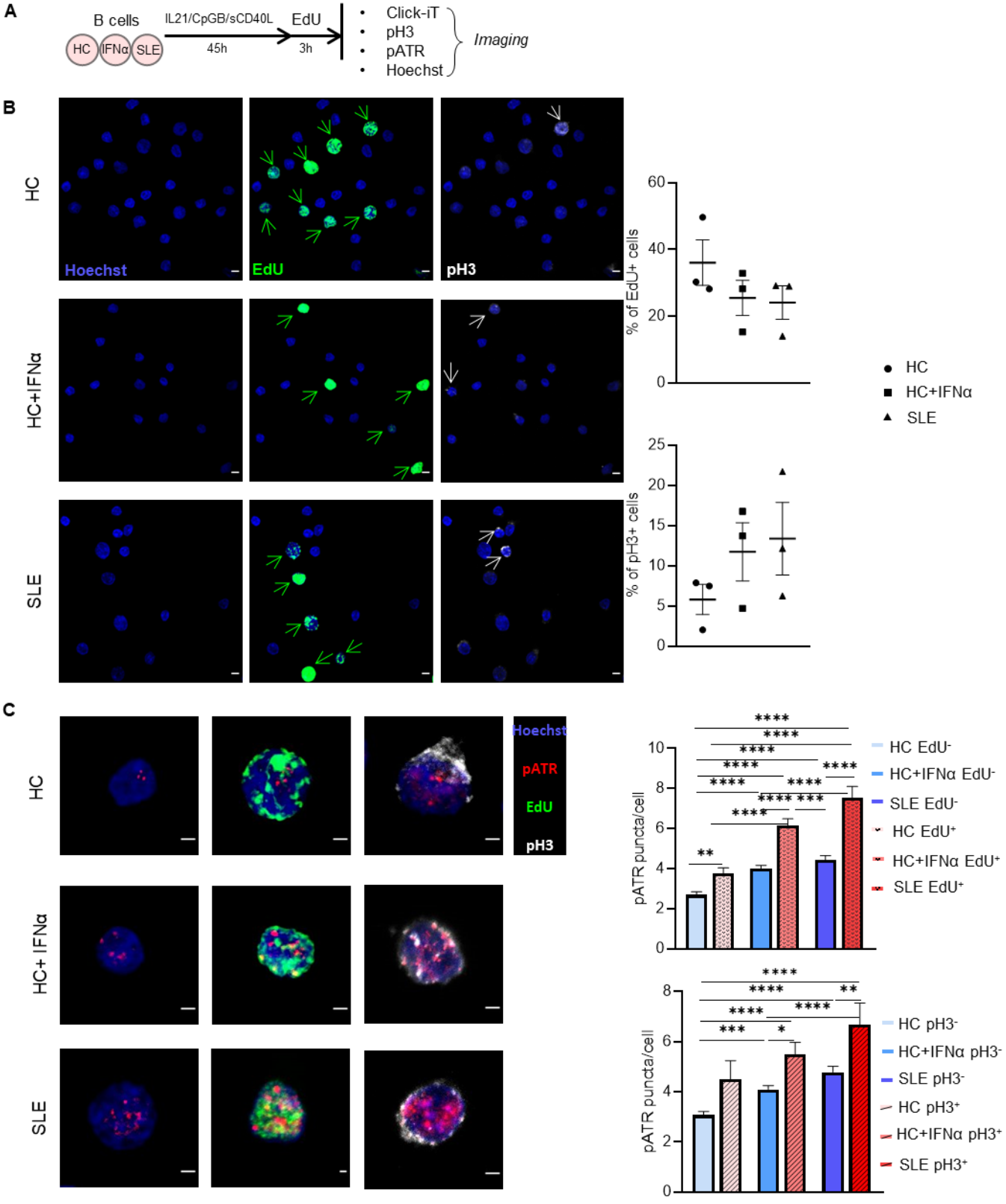

A
B cells
HC
IFNα
SLE
IL21/CpGB/sCD40L
45h
EdU
3h
Click-iT
pH3
pATR
Hoechst
Imaging
B
HC
HC+IFNα
SLE
Hoechst
EdU
pH3
% of EdU+ cells
% of pH3+ cells
HC
HC+IFNα
SLE
C
HC
HC+ IFNα
SLE
Hoechst
pATR
EdU
pH3
pATR puncta/cell
HC EdU-
HC+IFNα EdU-
SLE EdU-
HC EdU+
HC+IFNα EdU+
SLE EdU+
pATR puncta/cell
HC pH3-
HC+IFNα pH3-
SLE pH3-
HC pH3+
HC+IFNα pH3+
SLE pH3+

Figure 15. Increased pATR in replicative and mitotic SLE B cells. (A) B cells were isolated from HC and SLE patients (n=3 per group) using magnetic bead-based approach and were cultured for 45h with IL21/ CpGb/ sCD40L survival and mild proliferation stimuli in the presence or absence of IFNα (850U/ml) (in the case of HC), followed by 3 hours of EdU treatment and immunofluorescence staining as indicated in the schematic representation. **(B)** Representative confocal microscopy images and quantification of replicative (EdU+) and mitotic (pH3+) HC, HC+IFNα and SLE B cells. No statistical significance was observed (one-way ANOVA). **(C)** Representative confocal microscopy images and quantification of pATR in EdU-, EdU+, pH3- and pH3+ cells from HC, HC treated with IFNα and SLE B cells. Analysis for pATR staining was performed as puncta per cell using a macro developed in Fiji software (2). *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 (one-way ANOVA). Scale bar: 2µm. Results are presented as mean ± SEM.

Inhibition of ATR alters cytokine production of IFNα-treated B cells.

To clarify the functional involvement of ATR-mediated DDR mechanism in B cells in a *SLE-like* environment, total B cells were isolated from the periphery of healthy volunteers and were exposed to IFNα, the main type I IFN, following their *ex vivo* activation (IL-21/ CpGb/ sCD40L cocktail for survival and mild induction of proliferation) (158–166) **(Fig. 16A).** ATR mRNA, pATR and pChk1 protein levels were upregulated upon IFNα administration **(Fig. 16B-D)** whereas no significant alterations in ATM mRNA, pATM, pChk2 and pDNA-PKcs expression levels were noted **(Fig. 16B)**. Moreover, IFNα-treated B cells exhibited ATR pathway activation when being in S and G2/M phases of the cell-cycle similar to SLE B cells **(Fig. 15)**. Therefore, IFNα exposure of B cells recapitulated the cardinal feature of upregulated ATR-mediated DDR pathway noted in SLE being a sufficient experimental setup to mimic the SLE environment throughout this study.

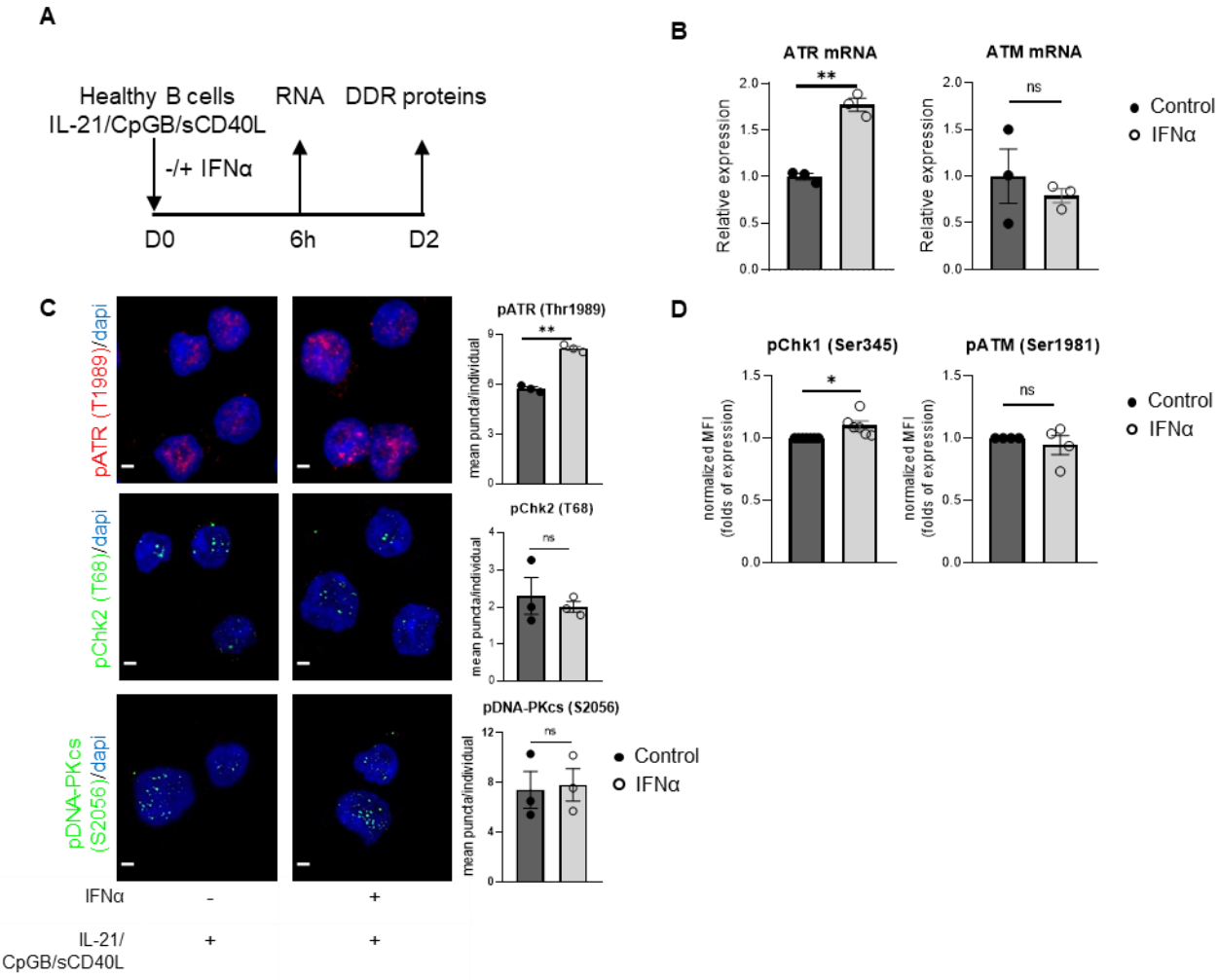


Figure 16. IFNα recapitulates DDR of SLE B cells. (A) Schematic representation of IFNα-treated B cell *ex vivo* experiments to mimic lupus environment. **(B)** Relative expression levels of ATR

and ATM mRNA in control (medium) and IFNα-treated conditions measured with quantitative real time RT-PCR (n=3 individuals per condition) (paired t-test). **(C)** Control (medium) and IFNα-treated B cells were stained and quantified (puncta/cell) for pATR (Thr1989) (red) and labeled with DAPI nuclear staining (blue) (n=3 individuals per condition) (paired t-test). Scale bar: 2μM. **(D)** Assessment of pChk1 (Ser345) in control (medium) and IFNα-treated B cells via flow cytometry followed by normalization of MFI to the MFI of control cells (unpaired t-test). Cells were gated on $CD19^{+}$. MFI: mean fluorescent intensity. *p<0.05, ** p<0.01.

To delineate the role of ATR in SLE pathogenesis, I first sought to examine the cytokine production following ATR inhibition in IFNα-treated B cells, considering that B cells are a rich source of various cytokines which are broadly perturbated in SLE (20). To date, whether ATR-mediated DDR pathway is involved in the production and secretion of cytokines remains ill-defined. To this end, berzosertib (ATRi), a pharmaceutical inhibitor that restrains ATR function by blocking Chk1 activation (167,168), was introduced to IFNα-treated healthy B cells *ex vivo* **(Fig. 17A, B)**. I then assessed the release of important cytokines (TNF-α, IL-13, IL-4, IL-10, IL-6, IL-2, TNF-β, IFN-γ, IL-17A, IL-12p70, APRIL, BAFF, CD40L) for both SLE pathology and B cell growth in IFNα-treated B cells with or without ATRi. Interestingly, IL-10, IL-6, IL-4 and TNF-β levels were decreased upon ATRi, while IL-12p70, IL-2 levels were increased, indicating a cytokine expression milieu that may alleviate SLE activity **(Fig. 17C)**. On the contrary, CD40L, BAFF, APRIL and IFNγ levels were also increased upon inhibition **(Fig. 17C)**, suggesting a hyperactive state where B cells are expected to grow, undergo immunoglobulin class switching (CSR), secrete antibodies and differentiate into antibody-secreting cells thus promoting SLE pathogenesis (169–174). Taken together, these data demonstrate that ATRi may reprogram the cytokine profile of SLE-like B cells.

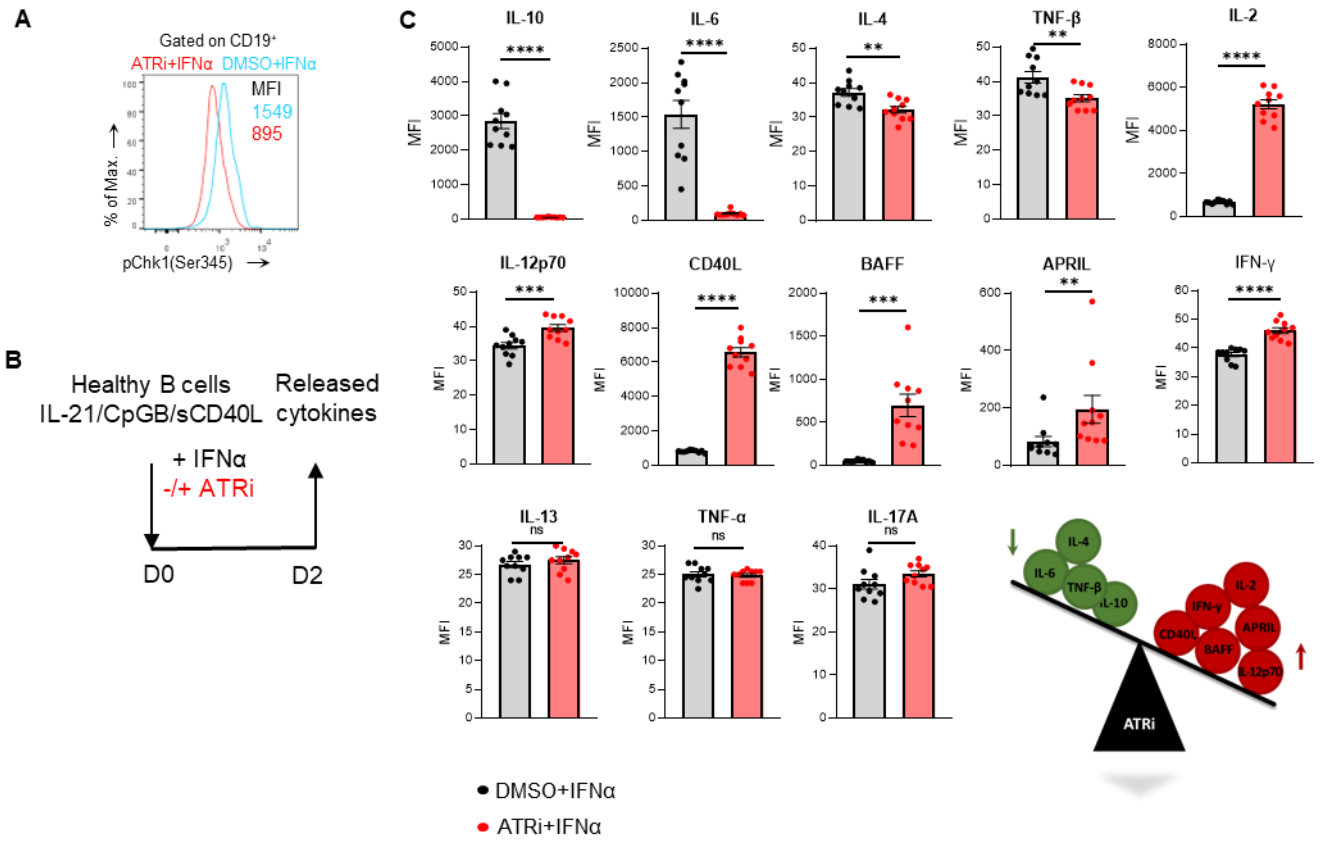


Figure 17. ATRi alters release of cytokines in IFNα-treated B cells. (A) Efficiency of ATRi berzosertib is demonstrated by the reduced levels pChk1 (Ser345 in IFNA treated B cells as depicted in the histogram **(B)** Schematic representation of ATRi experiment at IFNα-treated B cell *ex vivo* to assess cytokine production (ATRi→ berzosertib 5μM). **(C)** Detection of released cytokines (IL-10, IL-6, IL-4, TNF-β, IL-2) involved in B cell function, activation, proliferation and survival utilizing LEGENDplex™ technology through flow cytometry at day 2 (D2) of culture (n=10 individuals per condition) (paired t-test). MFI: mean fluorescent intensity. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Inhibition of ATR reduces the immunogenicity of IFNα-treated B cells.

Results thus far imply that ATR-mediated DDR pathway is critically involved in the production of cytokines by B cells in SLE, plausibly driving SLE pathophysiology in an autocrine fashion. Moreover, SLE B cells are known to adopt an activated status and enhanced antigen presentation ability followed by increased plasmablast formation and antibody production (13,15,175–177). To this end, I evaluated cell activation (CD40, CD80 and surface BAFF) and antigen-presentation capability (HLA-DR) across different timepoints during inhibition of ATR activity in IFNα-treated B cells **(Fig. 18A-C)**. Upon inhibition of ATR, activation and antigen presentation by HLA-DR were downregulated in IFNα-treated B cells, even when ATRi (i.e., berzosertib) was administered at low dosage **(Fig. 19)**.

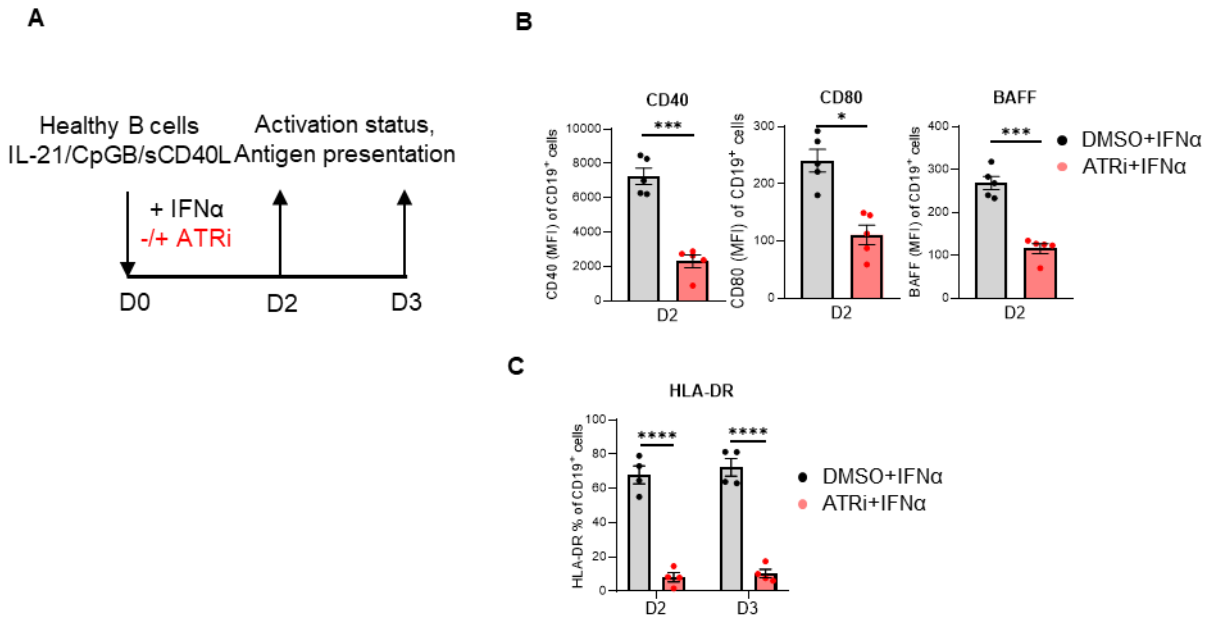


Figure 18. ATRi inhibits activation of IFNα-treated B cells. (A) Schematic representation of ATRi experiment at IFNα-treated B cells *ex vivo* for assessing cell activation status (ATRi→ berzosertib 5μM). Quantification of flow cytometry retrieved data of **(B)** CD40 (n=5 individuals per condition), CD80 (n=5 individuals per condition), BAFF (n=5 individuals per condition) (paired t-test) and **(C)** HLA-DR (n=3-4 individuals per condition). MFI: mean fluorescent intensity. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Of note, this phenotype may be ATR-dependent and Chk1-independent because pharmaceutical inhibition downstream of ATR-Chk1 interactive regulation with Chir-124 (Chk1i, a potent inhibitor of Chk1 activity) did not affect these cell properties **(Fig. 19, Fig. 20)**. Therefore, ATR activation *per se* plays a critical role in the activation of IFNα-treated B cells and, also, the aforementioned variable cytokine expression upon ATR inhibition does not reflect a hyperactive B cell population.

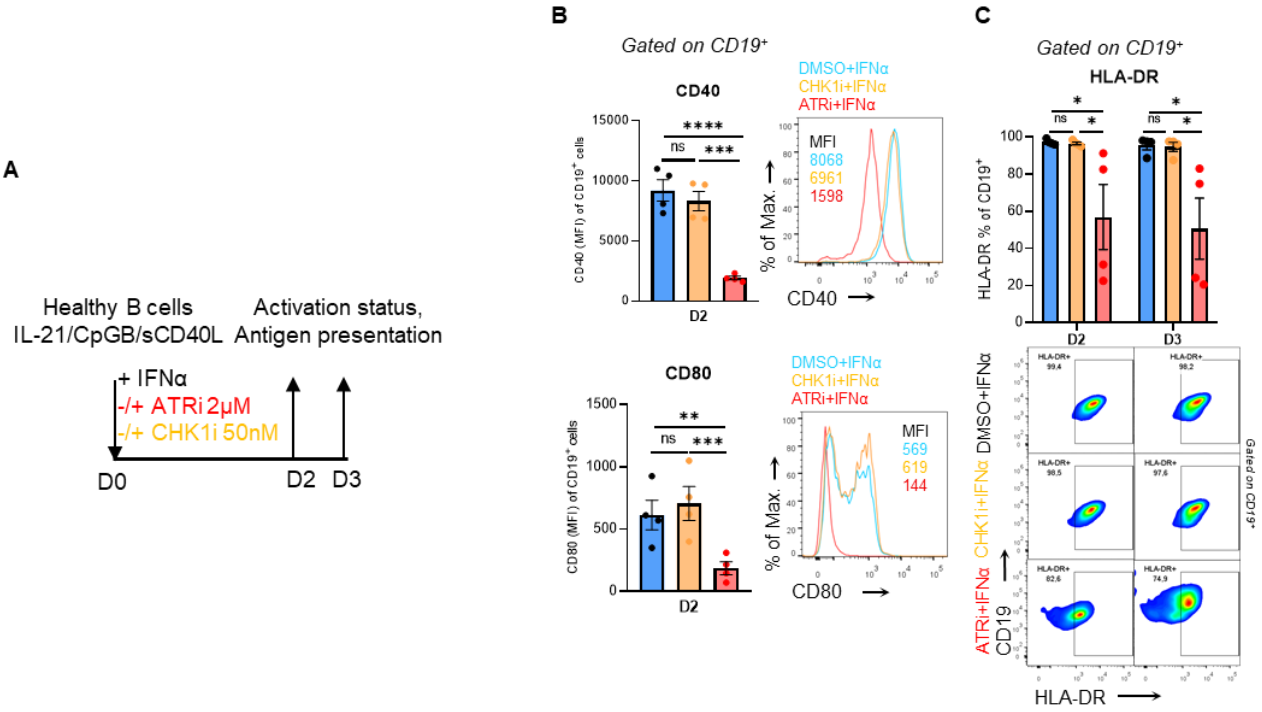


Figure 19. ATRi inhibits growth in IFNα-treated B cells even when administrated in a lower dose and this effect is specific of ATR perturbation and not of downstream Chk1. (A) Schematic representation of ATRi (berzosertib 2μM) *and CHK1i (CHIR-124→50nM)* experiment at IFNα-treated B cells *ex vivo* for assessing cell activation status. Quantification of flow cytometry retrieved data **(B)** CD40 (n=4 individuals per condition), CD80 (n=4 individuals per condition) (paired t-test), **(C)** HLA-DR (n=4 individuals per condition). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

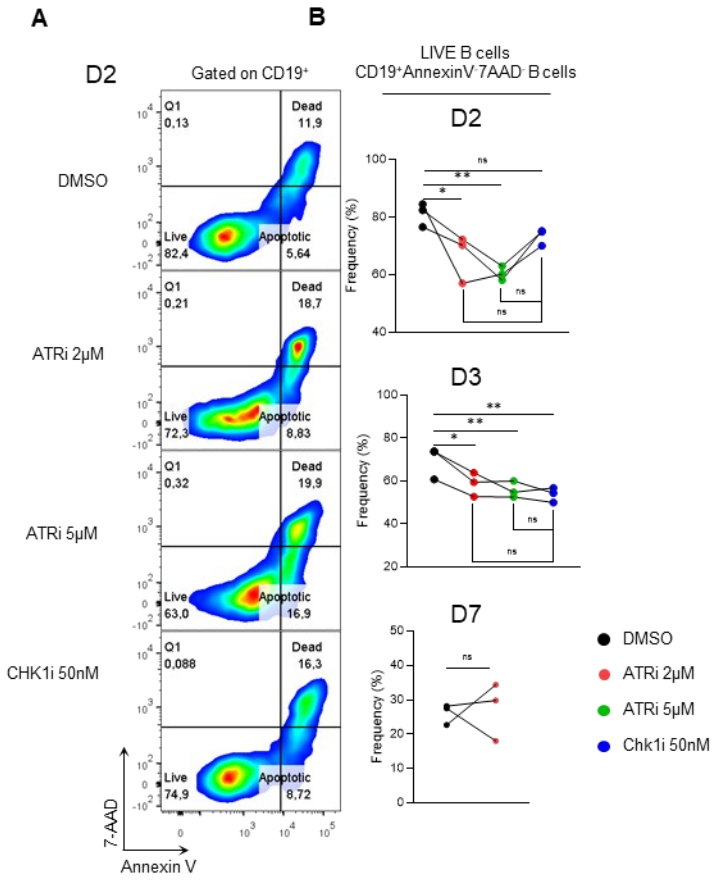


Figure 20. Evaluation of DDRi toxicity in B cells. (A) Representative flow cytometry plots for assessing live (CD19+AnnexinV-7AAD-), apoptotic (CD19+AnnexinV+7-AAD) and dead (CD19+AnnexinV+/7AAD+) B cells according to CD19, 7-AAD and Annexin V staining of isolated B cells upon DMSO, ATRi 2 µM, ATRi 5 µM and CHK1i 50 nM at day 2 (D2) of culture. All cultured B cells have been subjected to IFNα (850 U/ml) and IL-21/CpGB/sCD40L. **(B)** Viability analysis of B cells based on (A). N=3 individuals per condition. One-way repeated measures ANOVA. p≥0.05 (ns), *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Next, I investigated whether antibody (i.e., immunoglobulin) production is affected, since their production may follow the release of cytokines, as in the case of CD40L, BAFF and APRIL but, on the other hand, may also be attenuated due to decrease of pro-inflammatory cytokines, such as IL-6 and TNF-β. The results showed that surface IgM (sIgM) was decreased by day 3 of culture, surface IgD (sIgD) was decreased by day 7, whereas surface IgG (sIgG) did not exhibit any significant difference in IFNα-treated B cells exposed to ATRi compared to the unexposed **(Fig. 21A, B)**. Furthermore, released IgM, IgA, IgE, IgG1, IgG2, IgG3, IgG4 were strongly decreased in IFNα-treated

B cells with ATRi **(Fig. 21A, C),** suggesting that ATR DDR pathway has a central role in antibody formation.

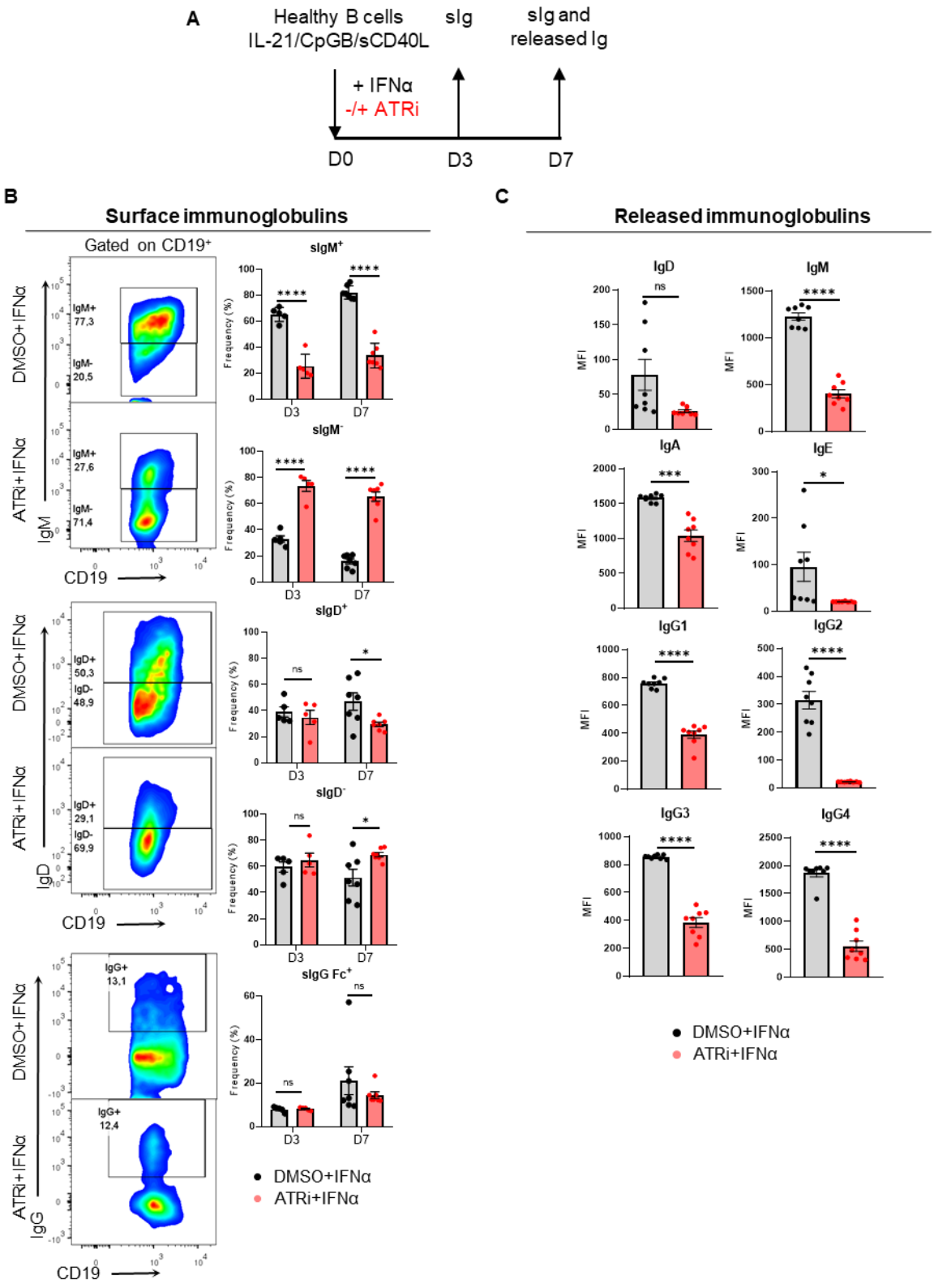


Figure 21. ATRi inhibits antibody formation in IFNα-treated B cells. (A) Schematic representation of ATRi experiment (ATRi→ berzosertib 2μM) at IFNα-treated B cell *ex vivo* to assess surface and released immunoglobulins. **(B)** Representative flow cytometry gatings for

IgM, IgD and IgG at day 7, and quantification of IgM, IgD and IgG of B cells at days 3 and 7 (n=5-7 individuals per condition) (two-way ANOVA). **(C)** Detection of released immunoglobulins (IgM, IgD, IgA, IgE, IgG1, IgG2, IgG3, IgG4) utilizing LEGENDplex™ technology through flow cytometry (n=8 individuals per condition) (paired t-test). MFI: mean fluorescent intensity. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Due to the decreased levels of both surface IgM, IgD and released immunoglobulins by IFNα-treated B cells with ATRi, a reduction in isotype-switched and antibody-secreting B cells was anticipated. Therefore, I next sought to investigate B cell subset formation **(Fig. 22A).** Indeed, at day 7 of culture, IFNα-treated B cells with ATRi exhibited dramatically decreased levels of isotype-switched memory (swme) cells ($CD19^+IgD^-CD27^+$), total memory cells ($CD19^+CD27^+$), transitional cells ($CD19^+IgD^{dim}CD38^+$) and plasmablasts ($CD19^+IgD^-CD27^+CD38^+$), while isotype-unswitched memory (unswme) ($CD19^+IgD^+CD27^+$), naïve ($CD19^+IgD^+CD27^-$) and double negative (DN) cells ($CD19^+IgD^-CD27^-$) remained almost unaffected compared to those not treated with ATRi **(Fig. 22A, B)**.

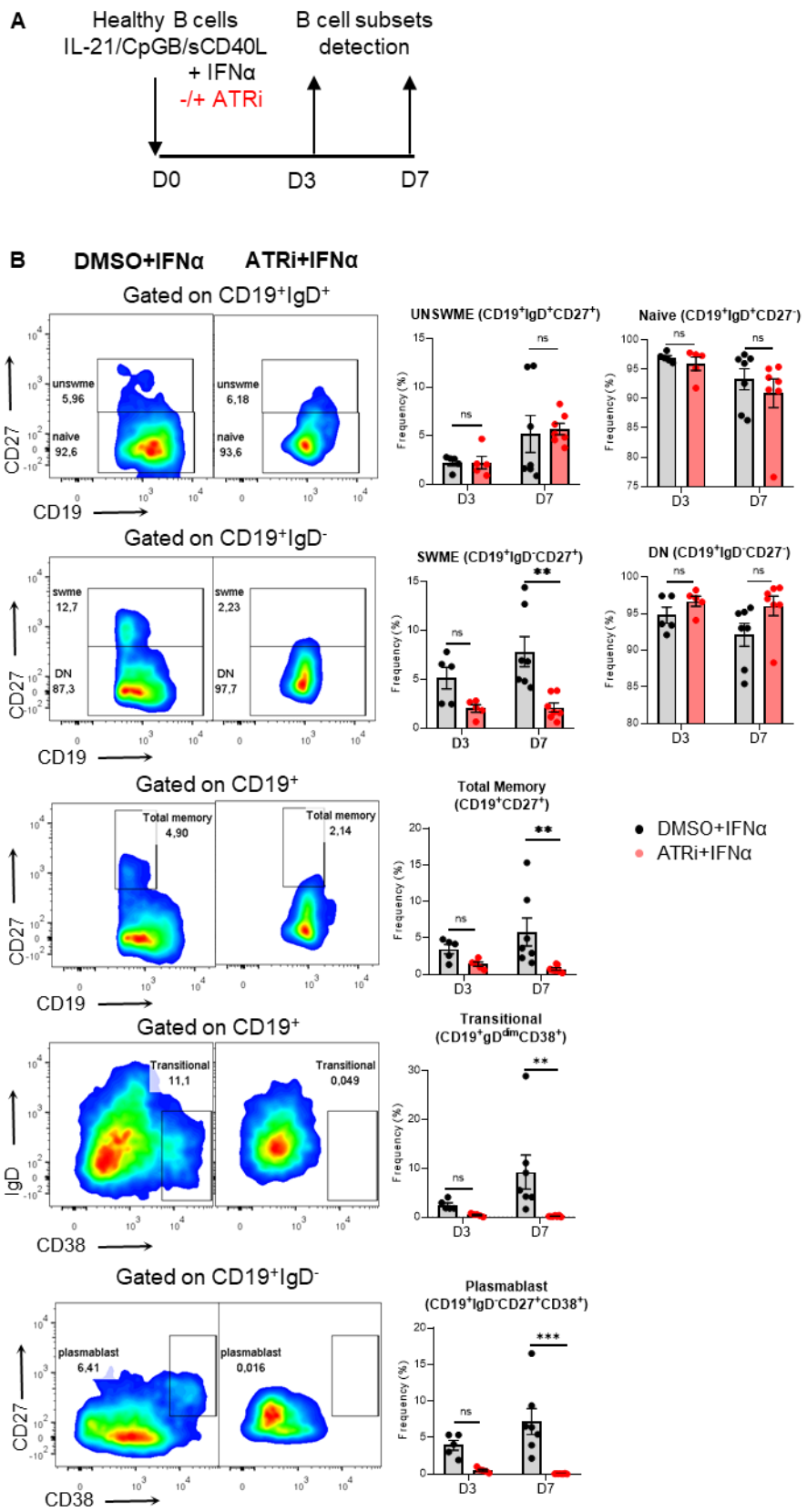


Figure 22. ATRi affects differentiation of IFNα-treated B cells. (A) Schematic representation of ATRi experiment (ATRi→ 71erzosertib 2μM) at IFNα-treated B cells *ex vivo* for assessing B cell subsets differentiation status. **(B)** Representative flow cytometry gated cell populations at day 7 and quantification of corresponding populations at days 3 and 7 (D3: n=5 individuals per condition, D7: n=7 individuals per condition) (two-way ANOVA). ** p<0.01, *** p<0.001.

Finally, I examined whether ATRi influences SLE B cell responses in additional ways such as by affecting the cell-cycle profile. Our data in IFNα-treated B cells showed that upon ATRi there were significantly increased G0-derived B cells compared to DMSO condition (control), an observation consistent with the reduced cell cluster formation as observed under the upright microscope **(Fig. 23)**. Therefore, by introducing the SLE-like B cells to a resting state (G0), their immune responses declined as expected.

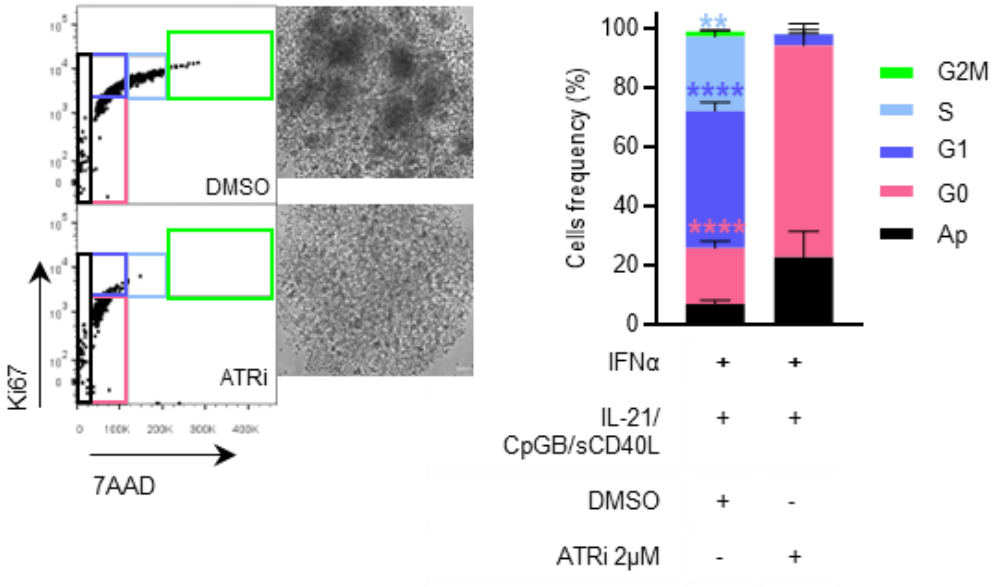


Figure 23. ATRi affects cell cycle progression of SLE-like B cells. B cells were isolated from healthy individuals (n=3) using magnetic bead-based approach and were cultured for 3 days with IFNα (850U/ml), IL21/ CpGb/ sCD40L survival and mild proliferation stimuli in the presence or absence of ATRi (2μM) or DMSO (control), followed by Ki67/7-AAD cell cycle analysis via flow cytometry. Linear scale was used for 7-AAD (DNA content). The comparison was done between the same cycle phase of the two conditions (ATRi and DMSO). G0, G1 and S phase -derived cells presented statistical significance between the two conditions. Representative flow cytometry plots and corresponding microscopy images. ** $p<0.01$, **** $p<0.0001$ (two-way ANOVA). Results are presented as mean ± SEM. Scale bar: 10μM.

Collectively, these results suggest that inhibition of ATR activity reduces the immunogenicity of IFNα-treated B cells by: a) decreasing the release of IL-10, IL-6, IL-4 and TNF-β levels, while increasing the release of IL-12p70, IL-2, b) attenuating cell

activation, c) restraining immunoglobulin formation, d) inhibiting class-switching and formation of plasmablasts and e) arresting them in resting state.

IRF1 directly interacts with the promoter sequence of ATR gene in IFNα-treated B cells and modulates ATR activity.

To investigate the molecular mechanism via which *SLE-like* B cells undergo direct regulation of ATR by IFNα, I performed chromatin immunoprecipitation (ChIP) experiments for binding sites of IRF1 on *ATR* regulatory regions, as predicted by *in silico* analysis (EPD database) **(Fig. 24A)**. Both ATR and IRF1 mRNA levels were increased in B cells of SLE patients **(Fig. 24B),** as well as in healthy B cells upon IFNα administration **(Fig. 24C)**. Following ChIP reactions with anti-IRF1 antibody or control IgG in IFNα-treated B cells, I identified an IRF1-binding event *(i.e., loc1)* on *ATR* gene promoter close to the transcription start site of *ATR* gene that was specifically enriched for anti-IRF1 reactions **(Fig. 24D)**. These data suggest that IRF1 may directly interact with *ATR* regulatory sequences to transcriptionally mediate its expression.

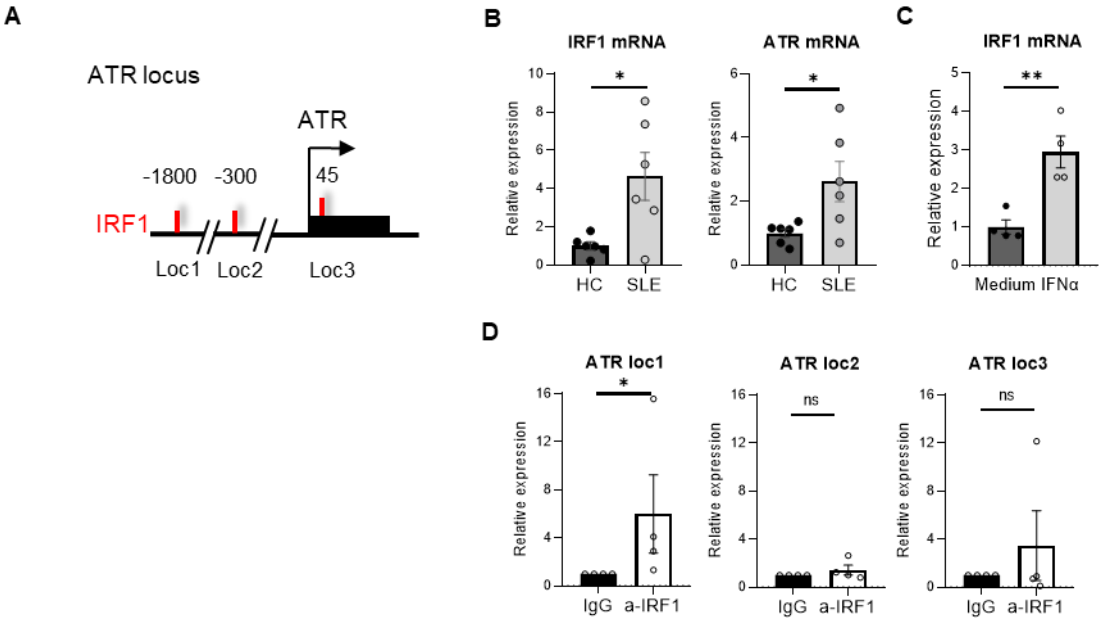


Figure 24. IRF1 directly interacts with the promoter sequence of ATR gene in IFNα-treated B cells. (A) Schematic representation of the ATR gene locus around the transcription start site (TSS: denoted with the broken arrow). The coding region of ATR gene is represented as a black box. Numbers above the schematic drawing denote the distance from the TSS and the predicted binding sites of IRF1 (EPD database). **(B)** IRF1 and ATR mRNA levels in B cells of SLE patients compared to HC (n=6 per condition, same samples for IRF1 and ATR) analyzed by real-

time RT qPCR using GAPDH normalization (unpaired t-test). **(C)** Administration of IFNα in B cells *ex vivo* promotes IRF1 mRNA expression as measured with quantitative real time RT-PCR (n=4 individuals per condition). **(D)** Chromatin immunoprecipitation (ChIP) analysis of the binding sites of IRF1 to ATR gene locus. ChIP experiments were performed using anti-IRF1 antibody (a-IRF1) or a control antibody (IgG) in chromatin isolated from B cells treated with IFNα. For a-IRF1 and IgG reactions the same amount of DNA was used as template. The primer pairs used to amplify the corresponding DNA sequences are indicated with specific loci numbers below the schematic drawing in (A). Note that IRF1 specifically binds to the locus 1 and less efficient to the other loci (n=4 individuals per condition). *p<0.05, ** p<0.01.

To directly assess whether IRF1 binding on *ATR* gene locus has indeed a regulatory role, I performed knockdown of IRF1 with small interfering IRF1 RNA (siIRF1) or negative control (siNeg) in IFNα-treated B cells and examined ATR mRNA, ATR, pATR (Thr1989) and pChk1 (Ser345) protein levels **(Fig. 25A)**. ATM mRNA levels were also checked as a control to ensure IRF1 specificity for ATR. Knockdown of IRF1 in these cells downregulated ATR mRNA, ATR, pATR and pChk1 protein levels, whereas retained ATM mRNA levels **(Fig. 25B, C, D)**. Importantly, IRF1 silencing suppressed the development of both transitional and plasmablast B cell subsets, similar to the results obtained following pharmaceutical ATR inhibition **(Fig. 25E).**

Overall, these results support that IRF1 mediates ATR activity explaining the DDR observed in IFNα-treated and SLE B cells.

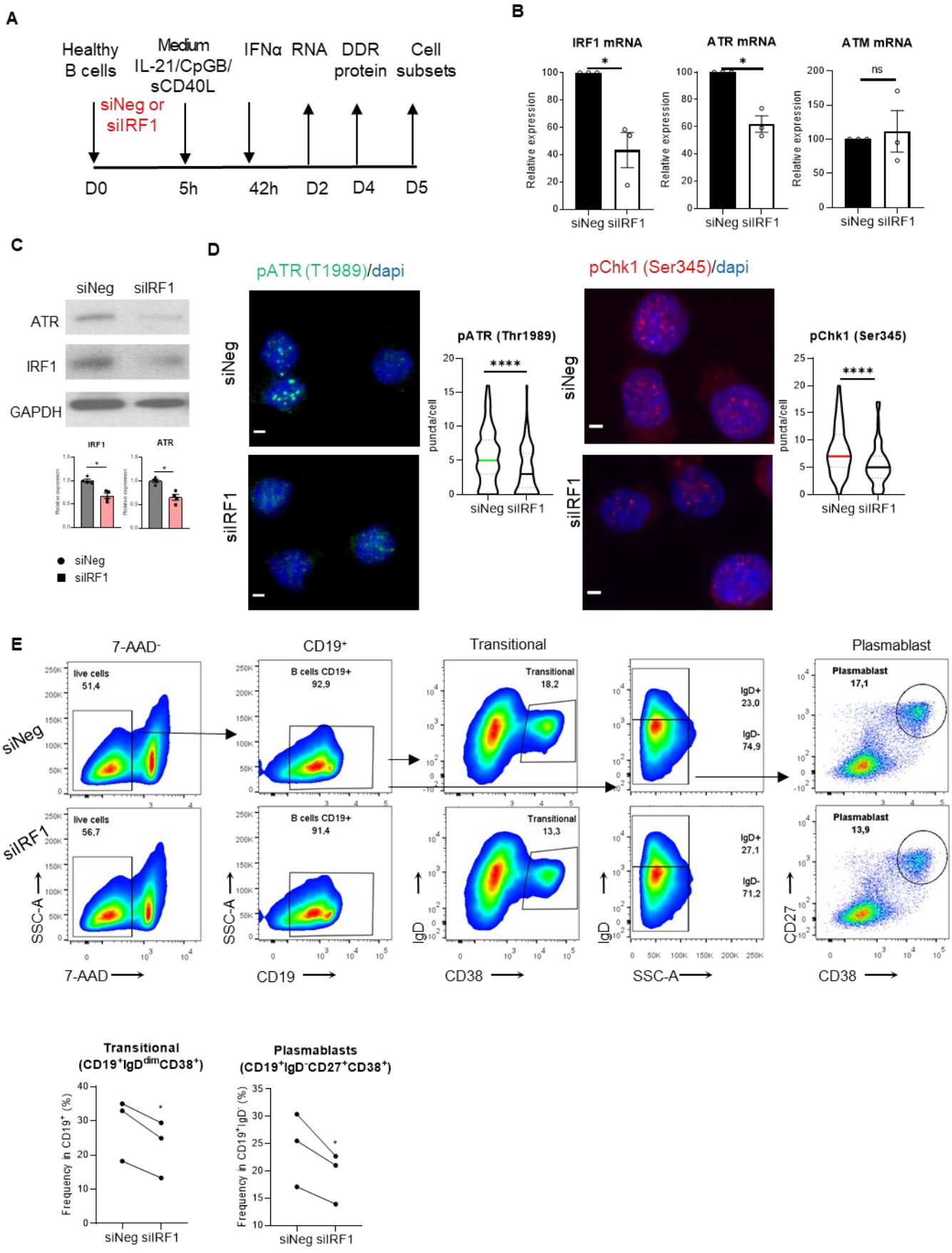

A
Healthy B cells
Medium IL-21/CpGB/ sCD40L
IFNα
RNA
DDR protein
Cell subsets
siNeg or siIRF1
D0
5h
42h
D2
D4
D5
B
IRF1 mRNA
ATR mRNA
ATM mRNA
Relative expression
siNeg siIRF1
ns
C
siNeg
siIRF1
ATR
IRF1
GAPDH
D
pATR (T1989)/dapi
pChk1 (Ser345)/dapi
pATR (Thr1989)
pChk1 (Ser345)
puncta/cell
****
E
7-AAD-
CD19+
Transitional
Plasmablast
live cells
51,4
56,7
B cells CD19+
92,9
91,4
Transitional
18,2
13,3
Plasmablast
17,1
13,9
IgD+
23,0
27,1
IgD-
74,9
71,2
SSC-A
7-AAD
CD19
CD38
IgD
CD27
Transitional
(CD19+IgDdimCD38+)
Plasmablasts
(CD19+IgD-CD27+CD38+)
Frequency in CD19+ (%)
Frequency in CD19+IgD- (%)

Figure 25. IRF1 directly modulates ATR activity in IFNα-treated B cells. (A) Schematic representation of silencing IRF1 expression in B cells *ex vivo* treated with IFNα using as siRNA siIRF1 or as a control siRNA siNeg in order to assess ATR signaling activity. **(B)** Relative expression levels of IRF1 (to assess efficiency of siIRF1), ATR and ATM mRNA of siNeg and siIRF1 conditions measured with quantitative real time RT-PCR (n=3 individuals per condition). **(C)** B cells were isolated from healthy individuals (n=4) using magnetic bead-based approach and were cultured ex vivo and transfected with siIRF1 or siNeg (scramble, control) for 42 hours, followed by IFNα and IL21/ CpGb/ sCD40L survival and mild proliferation stimuli exposure till day 4 where western blot analysis was performed with ATR and IRF1 antibodies. GAPDH blotting was also applied to confirm equal loading of each sample. Representative samples depicted. Results are presented as mean ± SEM. *p<0.05 (paired Student's t test). **(D)** SiNeg and siIRF1 B cells were immunostained and quantified (puncta/cell) for pATR (Thr1989) (green) or pChk1 (Ser345) (red) and labeled with DAPI (blue) (n=3 individuals per condition) (un-paired t-test). Scale bar: 2μM. **(E)** Analysis of flow cytometry retrieved data for transitional and plasmablast B cells upon siRNA (siNeg and siIRF1, n=3 individuals per condition) (ratio paired t-test). *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

DDR in the SLE-involved skin

Skin is one of the most affected organs in SLE. SLE with skin lesions can produce considerable morbidity resulting from painful skin lesions, alopecia, disfigurement, etc. To this end, I proceeded with the examination of DDR activation in lesional and non-lesional skin retrieved from SLE patients compared to skin from healthy individuals. Interestingly, immunofluorescence analysis of γH2AX indicated higher levels of DDR in lesional (n=3) compared to non-lesional (n=2) or healthy (n=2) skin **(Fig. 26)**. These observations suggest that aberrant DDR may be involved in the diseases affected organs.

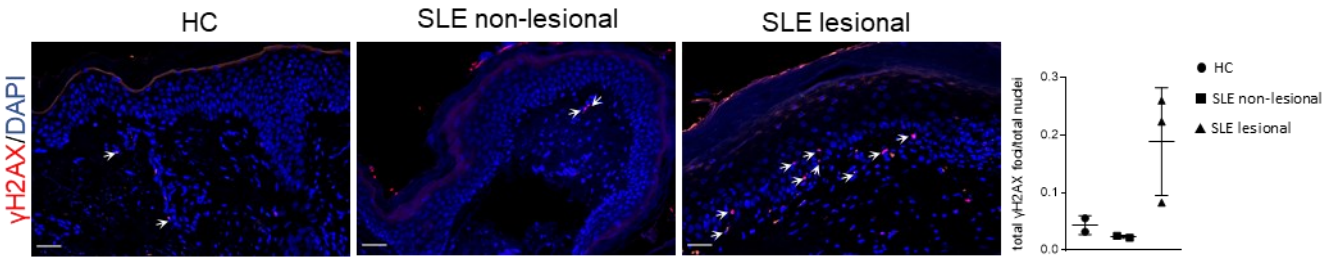


Figure 26. Increased DDR in lesional SLE skin. Representative confocal microscopy images for γH2AX (red) and nuclei (DAPI, blue) staining microscopy in the epidermis of HC (n=2), non lesional (n=2) and lesional SLE (n=3) samples. Scale bar: 40μM.

DISCUSSION

Although DDR has been implicated in all facets of inflammatory states and it may be triggered in affected tissues such as the skin little is known about how its deregulation may render immune cell responses pathogenic. Importantly, the DDR-driven molecular mechanisms in autoimmunity remain poorly understood. B cells have a pivotal role in the development of SLE disease, however the driving factors for their pathogenicity are elusive. In this study, using proteomic and transcriptomic analyses of B cells from patients with SLE, a deregulated DDR is revealed. More specifically, this thesis describes a novel mechanism associated with B cell dysfunction which entails the engagement of ATR-mediated pathway being specifically enriched in SLE B cells. Importantly, our *ex vivo* data revealed that in the course of an autoimmune response driven by type I IFN, ATR-mediated pathway was triggered and that targeted pharmaceutical inhibition of ATR activity restrained key features of SLE pathophysiology including B cell activation, plasmablast formation, antibody production and pro-inflammatory cytokines release. The present study provides evidence that ATR overactivation induced by type I IFN in B cells is mediated by direct molecular interaction with IRF1. Together, these data link for the first time the characteristic type I IFN signature of SLE with the upregulation of ATR-mediated pathway and the induction of pathogenic B cells mediated via IRF1. Pharmacologic targeting of ATR alleviates the pathogenic cell features pointing to the ATR pathway as a potential therapeutic target in SLE.

In accordance with the literature, upregulated DDR was also observed in SLE T cytotoxic and neutrophils (178,179), but B cells exhibited the most prominent DDR increase. Our data derive from both naïve and active-phase patients, suggesting that these findings are likely mirroring a primary pathogenetic mechanism in SLE. Although roles of DDR components have been previously proposed in pathogenic cells in processes associated with SLE- including their implications in V(D)J recombination, somatic hypermutation and cell death (66,77,180)- a comprehensive understanding of their specific contribution to autoreactive B cell responses is ill-defined. Here, this study expands this knowledge by identifying the ATR-mediated DDR pathway deregulation in B cells and links this pathway to their pathogenic potential. While our data highlight a clear increase in ATR/Chk1 pathway activation, with no alterations in

ATM/Chk2 and DNA-PKcs in SLE B cells, a study by Taher et al. reported a reduction in ATR activation and an increase in ATM activation in B cells from patients with SLE (181). In contrast to this study, our experimental design consisted of patients who had both high disease activity and were not under cytotoxic medication to avoid the possibility that the observed DDR may be affected by these drugs. In agreement with our findings, studies on B-cell lymphomagenesis-in the context of Epstein–Barr (EBV) virus infection- demonstrate the involvement of ATR/Chk1 in abnormal B cell responses (182–186). In particular, in a recent study (186) examining DDR activation in EBV-exposed B cells, ATR/Chk1- but not ATM/Chk2 pathway- was activated while specific targeting of ATR was able to suppress B cell aberrant function upon the infection. ATR promotes CSR in B cells and Chk1 expression is required for normal B cell growth and function, suggesting that although these two critical DDR components are closely co-regulated, they may also have distinct roles (187–189). SLE patients have increased risk of B-cell lymphoma, and EBV virus has been acknowledged as potential inducer of lupus autoimmunity, indicating that these conditions share common pathogenetic mechanisms (190). It is tempting to speculate that ATR/Chk1 deregulation could drive the abnormal B cell profile in these conditions. On the other hand, ATM/Chk2 pathway is of interest in B cell autoimmunity since it has been recently reported to drive B cell pathogenicity in a group of patients with RA exhibiting severe disease (83). ATM deregulation both depends and triggers IFN signaling following DDR perturbations and infections (191,192). These data coupled with our findings suggest that distinct DDR pathways may drive differential pathogenic responses of the same cell population depending on the type of the autoimmune environment.

Targeting B cells or B-cell expressing molecules have generated promising results in patients with SLE (3). The most recently approved therapies for SLE include the monoclonal antibody against B cell-activating factor (BAFF) and targeting type I IFN through a receptor monoclonal antibody that blocks the action of type I IFNs (IFN-α and -β) (193). These and other standard therapies for SLE (such as anti-CD20 drugs and cyclophosphamide) deplete most B cells including those involved in antiviral immunity, generating a major limitation of these therapies in the face of the current COVID-19 pandemic (194). SLE patients have a currently unmet medical need for more

effective and safer therapies suggesting that there is a missing link between the disease pathogenesis and the available therapies. I envisage that this link could be the aberrant DDR of B cells that is not affected by the current drugs. Targeting of ATR-mediated DDR pathway in SLE B cells could halt the pathogenic B cell responses, while potentially sparing the protective responses. In this direction, chemical compounds such as berzosertib could be encapsuled in nanoparticles conjugated with anti-CD19 to direct their uptake into CD19+ cells. Optimization of the drug concentration could allow manipulation of B cells overexpressing the pathway of interest (195–197).

Our data demonstrate that blockade of ATR activity in IFNα-treated B cells was followed by a B cell profile that does not predispose to SLE- such as the reduction in antibody formation, of $CD38^{+}$ B cells, of soluble IL-10, IL-6, IL-4, TNF-β and the increase of soluble IL-2- yet the released levels of BAFF were increased while membrane-bound BAFF decreased. Current anti-BAFF therapy, used in SLE, targets both the soluble form and the membrane-bound form of BAFF, with a higher potency for the soluble form (198,199). While forms of BAFF are biologically active, whether specific targeting of membrane-bound versus soluble BAFF would yield different clinical outcomes is not established. These findings highlight ATR as a potential therapeutic target for SLE and reveal an integral role of ATR DDR pathway in cytokine production by B cells. It is not clear whether this is a direct effect between ATR activity and signaling of cytokine production or its due to changes in B cell differentiation status. Rodier et al (39) demonstrated that ATM activity is essential for IL-6 production in fibroblast, however when ATR was inhibited, the production of IL-6 was diminished. Also, in a recent study (83), increased IL-6 secretion was associated with defects in ATM activation by human B cells in RA. Therefore, I conclude that in SLE B cells, cytokine release may be affected by aberrant ATR-mediated DDR and that the specific DDR signaling that modulates cytokine secretion varies depending on the cell type and the immune microenvironment.

Although our findings indicate that both ATR and Chk1 are excessively phosphorylated in SLE B cells compared to HC, the activation and antigen-presentation status of SLE-like B cells is affected only when ATR, but not Chk1, is inhibited. It is expected that ATR or Chk1 down-regulation should cause similar alterations, since Chk1 activation requires its phosphorylation by ATR. However, it is possible that these two

components may not always function as a linear pathway as supported by several studies presenting differences between the effects of ATRi and Chk1i (200–203). In this direction, there is evidence that ATR drives CSR while Chk1 is more involved in normal B cell growth (187,189,204) suggesting their distinct contribution to B cell physiology. Most interestingly, Speroni et al. (205) showed that Chk1, but not ATR, drives the progression of replication following UV irradiation of U2OS cells. An ATR-independent role of Chk1 mediated through its specific interaction with proliferating cell nuclear antigen (PCNA) has been also previously described (206,207) while Luciani et al. (202) have reported an ATR-dependent, Chk1-independent, intra-S-phase checkpoint that suppresses initiation of replication. Moreover, in a pioneering study (203), the authors have unveiled an ATR role to interact with RPA- a key sensor eliciting DDR following cellular exposure to genotoxic stresses (208)- independently of Chk1-mediated replication stress responses. Finally, the possibility of a different time-point dependent activation between ATR and Chk1 has been investigated, however it was not confirmed suggesting that these two components have distinct roles (200). Our results uncover a new mechanism of type I IFN contribution to SLE pathogenesis through induction of ATR-mediated DDR by IRF1 in B cells. In support of this, the findings: a) on B cells from patients with AS disease without an interferon signature exhibiting normal levels of ATR activation and b) on IFNα-treated B cells having increased levels of ATR activation, suggest that type I IFN is necessary for enriched ATR activity. IRF1 has been noted among the potential drivers of common B-cell lymphoid neoplasms and interacts with MUM1 which has a role in the progression of B-cell lymphoma (209–212). According to EPD database, other molecules than IRF1 may also directly interact with ATR regulatory regions suggesting that further investigation may reveal additional mechanisms driving ATR pathway in autoreactive B cells in B-cell mediated diseases.

In our study, IFNα-treated B cells fail to differentiate sufficiently towards plasmablast upon ATR or IRF1 inhibition suggesting that IRF1-ATR axis is critical for the plasmablast formation. In SLE, plasmablast population is often markedly expanded, and correlates with disease activity and flares (15). Depletion of plasmablast/plasma cells is currently used in SLE therapeutic interventions (3). Whereas an involvement of IRF1 has been recently reported in B cells that have differentiated towards plasma cells following LPS

stimulation (213), a specialized role of ATR in B cell lineage has not been described. Together, these findings suggest that the IRF1-ATR axis is important for B cell differentiation, and has a role in the aberrant plasmablast formation in SLE. Analysis in disease-involved tissues such as lesional skin indicated higher levels of DNA damage when compared to non-lesional or healthy skin. Collectively, these data underline the putative role of DNA damage in SLE and support further investigation of DDR molecular mechanisms to unravel its overall impact on the pathogenesis of SLE.

In conclusion, this thesis reports a specific DDR pathway being overactivated in B cells of SLE patients, one of the most overreactive cell population in SLE. I show that ATR-mediated DDR signaling, a critical response mechanism following genome instability, is abnormally regulated in B cells by IRF1, driving pathogenic cell responses. While relevant to understanding SLE pathogenesis and B-cell mediated autoimmune diseases, this may also provide novel insights into the specific regulatory role of ATR-mediated DNA damage response signaling in autoimmunity and into the coordination of ATR by interferon signaling. Overall our findings propose that targeted manipulation of IRF1-ATR axis in *SLE-like* B cells may be of therapeutic benefit in SLE. To this end, McNally et al. (71) have proposed the use of chemical DDR inhibitors to suppress immune responses in antigen-activated T cells in the human autoimmune diseases of hemophagocytic lymphohistiocytosis (HLH) and multiple sclerosis (MS). In this direction, various selective ATR inhibitors have been tested in phase I/II clinical trials for treating solid tumors (167,214). Importantly, VE-822, marketed as berzosertib has shown acceptable safety profile and early efficacy; currently being under evaluation in 18 ongoing clinical trials (e.g., NCT04266912, NCT03641313, NCT04052555).

Limitations of study related to human health

Targeting of ATR-mediated DDR pathway on pathogenic B cells may ameliorate autoimmune responses but at the same time compromise the immune responses to pathogens although this may be overcome at least in part with vaccination using approved vaccines. Moreover, therapeutic implementation of our findings may be hampered by the lack of protocols to specifically target the ATR pathway on

autoreactive B cells as opposed to broad B cell-directed inhibition. For this reason, to further explore the translational potential of the present findings, we propose to selectively target ATR activity in B cells both in vitro in primary cell cultures and in vivo in murine SLE models to examine the potential to be used for a clinical trial with SLE patients. To this end, we plan to use engineered exosomes that selectively target B cells and release berzosertib upon exosome uptake by the cells. Furthermore, generating an appropriate *ex vivo* experimental pipeline with patients-derived autoreactive B cells, retaining their disease-signature, where ATRi effects may be investigated, is an important matter of future studies.

TABLES

Table 1. Characteristics of SLE patients whose peripheral blood samples where utilized.

SLE SUBJECT	AGE	SEX	SLEDAI	PGA	Organ Involvement	Treatment at the time of sampling
SLE-1	43	F	14	3	LN	Off treatment 5 months prior to enrollment
SLE-2	37	F	12	2.5	NPSLE - Chorea	MTX, HCQ
SLE-3	59	M	12	2.5	NPSLE - Movement disorder	HCQ
SLE-4	38	F	10	3	LN	HCQ, AZA
SLE-5	52	F	14	3	LN	HCQ, BEL
SLE-6	44	F	12	3	NPSLE - Demyelination	HCQ
SLE-7	47	F	16	2.5	NPSLE	Naïve
SLE-8	60	F	14	2.5	LN-NPSLE psychosis	Naïve
SLE-9	28	M	12	2	Masckuloskeletal	HCQ
SLE-10	59	F	8	1.5	Masckuloskeletal	HCQ
SLE-11*	59	M	10	2	LN	Naïve
SLE-12*	56	M	14	2.5	Lung	Naïve
SLE-13*	35	M	15	3	LN-NPSLE	Naïve
SLE-14*	17	F	12	1.8	Masckuloskeletal	Off treatment 4 months prior to enrollment
SLE-15*	75	F	14	2.5	LN	Naïve
SLE-16*	25	F	14	2.5	LN	Naïve
SLE-17*	66	F	10	2	Cytopenia	MTX, HCQ

SLE-18*	46	F	14	2.5	NPSLE (ACS)	AZA
SLE-19*	47	F	14	2.5	LN	HCQ
SLE-20*	50	F	18	2.5	LN	Naïve
SLE-21*	19	F	10	2	LN	Naïve
SLE-22	26	F	14	2.5	Serositis-Cytopenia	Naïve
SLE-23	33	F	14	2.5	LN	Off treatment 18 months prior to enrollment
SLE-24	34	F	10	1.5	Masckuloskeletal	HCQ
SLE-25	35	F	10	2	Cytopenia	Naïve
SLE-26	42	F	22	3	LN	HCQ
SLE-27	44	F	8	3	NPSLE (Status E)	HCQ
SLE-28	52	F	14	2.5	LN	Naïve
SLE-29	50	M	10	3	LN-Serositis	Naïve
SLE-30	43	F	9	2.5	Cytopenia	Naïve
SLE-31	18	F	14	2.5	LN	HCQ
SLE-32	42	F	14	3	NPSLE (Myelitis)	HCQ
SLE-33	65	F	10	2	LN	Off treatment 3 months prior to enrollment
SLE-34	15	M	14	3	NPSLE	Naïve
SLE-35	44	F	10	2	LN	HCQ
SLE-36	43	F	14	2.5	NPSLE	Naïve
SLE-37	38	M	21	2.5	LN	Naïve
SLE-38	25	F	14	2.5	LN	HCQ, BEL, AZA
SLE-39	40	F	14	2.5	SLE	HCQ, GCs
SLE-40	49	F	14	3	NPSLE	HCQ, MTX
SLE-41	44	F	10	2	LN	Naïve
SLE-42	20	F	14	3	NPSLE	Naïve
SLE-43	24	F	14	2	LN	Naïve
SLE-44	45	M	12	2	LN	Naïve

SLE-45	76	F	14	2.5	LN	Naïve
SLE-46	29	F	10	1.5	LN	HCQ
SLE-47	53	F	20	3	LN	Naïve
SLE-48	67	F	14	2	NPSLE	Naïve
SLE-49	45	F	14	2.5	Serositis-Cytopenia	Naïve
SLE-50	47	F	14	2	LN	Naïve
SLE-51	44	F	10	3	Hemolytic anemia	Naïve (off treatment 2 years prior to enrollment)

Notes: SLE: Systemic lupus erythematosus; F: female; M: male; LN: Lupus nephritis; NPSLE: Neuropsychiatric lupus; ACS: acute coronary syndrome; MTX: methotrexate; HCQ: hydroxychloroquine; AZA: Azathioprine; BEL: Belimumab, GCs: Glucocorticoids, *Proteomics.

Table 2. Characteristics of AS patients whose peripheral blood samples where utilized.

AS SUBJECT	**AGE**	**SEX**
AS-1	40	F
AS-2	23	M
AS-3	40	M
AS-4	55	M

Notes. AS: axial spondylitis; F: female; M: male

Table 3. Characteristics of healthy individuals whose peripheral blood samples where utilized.

HEALTHY SUBJECT	**AGE**	**SEX**
HC-1	42	F
HC-2	56	M
HC-3	38	M
HC-4	26	M
HC-5	27	M
HC-6	40	F
HC-7	56	F
HC-8	53	F

HC-9	20	F
HC-10	45	F
HC-11	51	F
HC-12	28	M
HC-13	27	F
HC-14	32	F
HC-15*	56	M
HC-16*	63	M
HC-17*	18	F
HC-18*	35	M
HC-19*	50	F
HC-20*	25	F
HC-21*	49	F
HC-22*	26	F
HC-23*	56	F
HC-24*	60	F
HC-25*	52	F
HC-26	27	F
HC-27	32	F
HC-28	33	F
HC-29	46	F
HC-30	52	F
HC-31	34	F
HC-32	46	F
HC-33	43	M
HC-34	48	F
HC-35	25	F
HC-36	38	F
HC-37	58	F
HC-38	21	M
HC-39	57	M
HC-40	45	F

HC-41	31	M
HC-42	37	M
HC-43	28	M
HC-44	25	F
HC-45	27	M
HC-46	36	M
HC-47	22	M
HC-48	27	M
HC-49	24	F
HC-50	19	M
HC-51	23	M
HC-52	60	M
HC-53	52	M
HC-54	51	M
HC-55	31	M
HC-56	39	M
HC-57	42	M
HC-58	25	M
HC-59	58	M
HC-60	35	M
HC-61	27	F
HC-62	37	F
HC-63	20	M
HC-64	31	F
HC-65	33	M
HC-66	41	M
HC-67	52	M
HC-68	30	F
HC-69	36	M
HC-70	40	F
HC-71	24	F
HC-72	27	F

HC-73	25	M
HC-74	39	M
HC-75	42	F
HC-76	44	F
HC-77	51	F
HC-78	28	F
HC-79	31	F
HC-80	40	F
HC-81	50	F
HC-82	45	F
HC-83	37	F
HC-84	34	M
HC-85	36	F
HC-86	31	F
HC-87	53	F
HC-88	50	F
HC-89	28	F
HC-90	24	F
HC-91	24	F
HC-92	34	F
HC-93	35	F
HC-94	29	M
HC-95	21	F
HC-96	32	M
HC-97	46	F
HC-98	22	F
HC-99	40	F
HC-100	27	M
HC-101	39	M
HC-102	48	M
HC-103	57	F

Notes. HC: Healthy control; F: female; M: male; *Proteomics.

Table 4. Characteristics of volunteers (healthy or SLE) whose skin biopsies where utilized.

SUBJECT	AGE	SEX	SLEDAI	PGA	Organ involvement	Treatment at the time of sampling	Skin biopsy
sHC-1	60	M	NA		NA	NA	HC
sHC-2	23	M	NA		NA	NA	HC
sSLE-1	60	F	2	2	skin	Cyclophosmide	L, NL
sSLE-2	44	F	6	2	skin	Thalidomide	L, NL
sSLE-3	32	F	8	2.5	skin	Ustekinumab	L

Notes. sHC: skin Healthy control; sSLE: skin Systemic Lupus Eruthematosus; F: female; M: male; CYC: Cyclophosphamide; L: lesional; NL: non-lesional

Table 5. Antibodies used for flow cytometric analysis and immunostaining of immune cells.

Target	Fluorochrome	Clone	Vendor	Cat.	Application	Dilution
CD19	FITC	HIB19	Biolegend	302206	FC	1/100
CD19	PE-Cy7	HIB19	Biolegend	302216	FC	1/100
CD19	BV510	HIB19	Biolegend	302241	FC	1/100
CD19	Pacific Blue	HIB19	Biolegend	302224	FC	1/100
CD19	BV605	HIB19	Biolegend	302244	FC	1/100
CD4	PerCP-Cy5.5	OKT4	Biolegend	317428	FC	1/100
CD8	APC-Cy7	SK1	Biolegend	344714	FC	1/100
CD14	PE	18D11	ImmunoTools	21620144	FC	1/100
CD16	BV421	3G8	Biolegend	302037	FC	1/100
CD16	APC	3G8	Biolegend	302012	FC	1/100
CD66b	APC	G10F5	Biolegend	305118	FC	1/100
HLA-DR	APC-Cy7	L243	Biolegend	307618	FC	1/100
HLA-DR	Alexa Fluor 488	LN3	Biolegend	327014	FC	1/100
CD25	FITC	BC96	Biolegend	302604	FC	1/100
CD27	BV650	O323	Biolegend	302828	FC	1/100
IgD	BV785	IA6-2	Biolegend	348242	FC	1/100
CD38	FITC	HB-7	Biolegend	356610	FC	1/100
CD80	PerCP-Cy5.5	2D10	Biolegend	305232	FC	1/100

IgM	PerCP-Cy5.5	MHM-88	Biolegend	314512	FC	1/100
IgG Fc	PE-Cy7	M1310G05	Biolegend	410722	FC	1/100
BAFF	APC-Cy7	1D6	Biolegend	366512	FC	1/100
CD40	PE-Cy7	5C3	Biolegend	334322	FC	1/100
Foxp3	alexa fluor 647	259D	Biolegend	320214	FC	1/50
γH2AX (ser139)	PE-Cy7	2F3	Biolegend	613420	FC	1/50
phospho-ATM (ser1981)	PE	10H11.E12	Biolegend	651204	FC	1/50
cleaved PARP1 (Asp214)	PE	QA17A17	Biolegend	669904	FC	1/50
phospho-Chk1 (ser345)	PE	133D3	Cell Signaling	12268	FC	1/50
Ki67	PE	Ki-67	Biolegend	350504	FC	1/50
B220/CD45R	FITC	RA3-6B2	Biolegend	103206	FC	1/200
γH2AX (ser139)	unconjugated	JBW301	Millipore	05-636	IF	1/200
phospho-ATR (Thr1989)	unconjugated	polyclonal	GeneTex	GTX128145	IF, WB	1/100, 1/500
phospho-Chk1 (ser345)	unconjugated	133D3	Cell Signaling	2348S	IF, WB	1/50, 1/700
phospho-p53 (S15)	unconjugated	polyclonal	abcam	ab1431	IF	1/100
phospho-Chk2 (T68)	unconjugated	polyclonal	abcam	ab85743	IF	1.2/100
p95/NBS1	unconjugated	D6J5I	Cell Signaling	14956	IF	1/100

Cleaved Caspase-3 (Asp175)	unconjugated	polyclonal	Cell Signaling	9661S	IF	1/600
mouse IgG	Alexa fluor 555	polyclonal	Invitrogen	A-21425	IF	1/500
rabbit IgG	Alexa fuor 488	polyclonal	Invitrogen	A-11008	IF	1/400
rabbit IgG	CF® 555	polyclonal	Biotium	20033	IF	1/2000
rabbit IgG	Alexa fuor 647	polyclonal	Invitrogen	A-21246	IF	1/200
Phospho-Histone H3 (Ser10) (pH3)	unconjugated	K.872.3	Invitrogen	MA5-15220	IF	1/100
DNA PKcs (phospho S2056) (pDNA PKcs)	unconjugated	EPR5670	abcam	ab124918	IF	1/150
IRF-1 XP® Rabbit mAb	unconjugated	D5E4	Cell Signaling	8478S	ChIP, WB	1/50, 1/800
Rabbit mAb IgG XP® Isotype Control	unconjugated	DA1E	Cell Signaling	3900S	ChIP	1/200
ATR	unconjugated	E1S3S	Cell Signaling	13934S	WB	1/700
Chk1	unconjugated	2G1D5	Cell Signaling	2360S	WB	1/700
Actin	unconjugated	C4	merckmillipore	MAB1501	WB	1/5000
GAPDH	unconjugated	6C5	Invitrogen	AM4300	WB	1/1000

Rabbit Anti-Mouse IgG Antibody	HRP	polyclonal	Millipore	AP160P	WB	1/5000
Anti-rabbit IgG	HRP	polyclonal	Cell Signaling	7074S	WB	1/2000

Notes. FC: Flow Cytometry; IF: Immunofluorescence; WB: Western Blot; ChIP: Chromatin Immunoprecipitation

REFERENCES

1. Papadimitraki ED, Choulaki C, Koutala E, Bertsias G, Tsatsanis C, Gergianaki I, et al. Expansion of toll-like receptor 9-expressing B cells in active systemic lupus erythematosus: implications for the induction and maintenance of the autoimmune process. Arthritis Rheum. 2006 Nov;54(11):3601–11.

2. Yuen GJ. Autoimmunity in women: an eX(a)mination of eX(i)sting models. Clin Immunol [Internet]. 2019/10/24. 2020 Jan;210:108270. Available from: https://pubmed.ncbi.nlm.nih.gov/31669190

3. Fanouriakis A, Tziolos N, Bertsias G, Boumpas DT. Update on the diagnosis and management of systemic lupus erythematosus. Ann Rheum Dis. 2021 Jan;80(1):14–25.

4. Bertsias GK, Salmon JE, Boumpas DT. Therapeutic opportunities in systemic lupus erythematosus: state of the art and prospects for the new decade. Ann Rheum Dis. 2010 Sep;69(9):1603–11.

5. Tsokos GC. Systemic Lupus Erythematosus. N Engl J Med [Internet]. 2011 Nov 30;365(22):2110–21. Available from: https://doi.org/10.1056/NEJMra1100359

6. Bertsias GK, Nakou M, Choulaki C, Raptopoulou A, Papadimitraki E, Goulielmos G, et al. Genetic, immunologic, and immunohistochemical analysis of the programmed death 1/programmed death ligand 1 pathway in human systemic lupus erythematosus. Arthritis Rheum. 2009 Jan;60(1):207–18.

7. Nakou M, Knowlton N, Frank MB, Bertsias G, Osban J, Sandel CE, et al. Gene expression in systemic lupus erythematosus: bone marrow analysis differentiates active from inactive disease and reveals apoptosis and granulopoiesis signatures. Arthritis Rheum. 2008 Nov;58(11):3541–9.

8. Nakou M, Bertsias G, Stagakis I, Centola M, Tassiulas I, Hatziapostolou M, et al. Gene network analysis of bone marrow mononuclear cells reveals activation of multiple kinase pathways in human systemic lupus erythematosus. PLoS One. 2010 Oct;5(10):e13351.

9. Stagakis E, Bertsias G, Verginis P, Nakou M, Hatziapostolou M, Kritikos H, et al. Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. Ann Rheum Dis. 2011 Aug;70(8):1496–506.

10. Manzi S, Sánchez-Guerrero J, Merrill JT, Furie R, Gladman D, Navarra S V, et al. Effects of belimumab, a B lymphocyte stimulator-specific inhibitor, on disease activity across multiple organ domains in patients with systemic lupus erythematosus: combined results from two phase III trials. Ann Rheum Dis. 2012 Nov;71(11):1833–8.

11. Tsokos GC. Autoimmunity and organ damage in systemic lupus erythematosus. Nat Immunol [Internet]. 2020;21(6):605–14. Available from: https://doi.org/10.1038/s41590-020-0677-6

12. Allen ME, Rus V, Szeto GL. Leveraging Heterogeneity in Systemic Lupus Erythematosus for New Therapies. Trends Mol Med [Internet]. 2021;27(2):152–71. Available from: https://www.sciencedirect.com/science/article/pii/S1471491420302562

13. Scharer CD, Blalock EL, Mi T, Barwick BG, Jenks SA, Deguchi T, et al. Epigenetic programming underpins B cell dysfunction in human SLE. Nat Immunol. 2019;20(8):1071–82.

14. Yang J, Yang X, Wang L, Li M. B cells control lupus autoimmunity by inhibiting Th17 and promoting Th22 cells. Cell Death Dis [Internet]. 2020;11(3):164. Available from: https://doi.org/10.1038/s41419-020-2362-y

15. Karrar S, Cunninghame Graham DS. Abnormal B Cell Development in Systemic Lupus Erythematosus: What the Genetics Tell Us. Arthritis Rheumatol (Hoboken, NJ). 2018 Apr;70(4):496–507.

16. Domeier PP, Chodisetti SB, Schell SL, Kawasawa YI, Fasnacht MJ, Soni C, et al. B-Cell-Intrinsic Type 1 Interferon Signaling Is Crucial for Loss of Tolerance and the Development of Autoreactive B Cells. Cell Rep. 2018 Jul;24(2):406–18.

17. Banchereau J, Pascual V. Type I Interferon in Systemic Lupus Erythematosus and Other Autoimmune Diseases. Immunity [Internet]. 2006;25(3):383–92. Available from: https://www.sciencedirect.com/science/article/pii/S1074761306003955

18. Gottschalk TA, Tsantikos E, Hibbs ML. Pathogenic Inflammation and Its Therapeutic Targeting in Systemic Lupus Erythematosus. Front Immunol [Internet]. 2015 Oct 28;6:550. Available from: https://pubmed.ncbi.nlm.nih.gov/26579125

19. Parodis I, Stockfelt M, Sjöwall C. B Cell Therapy in Systemic Lupus Erythematosus: From Rationale to Clinical Practice [Internet]. Vol. 7, Frontiers in Medicine . 2020. p. 316. Available from: https://www.frontiersin.org/article/10.3389/fmed.2020.00316

20. Chan VS-F, Tsang HH-L, Tam RC-Y, Lu L, Lau C-S. B-cell-targeted therapies in systemic lupus erythematosus. Cell Mol Immunol [Internet]. 2013/01/28. 2013 Mar;10(2):133–42. Available from: https://pubmed.ncbi.nlm.nih.gov/23455017

21. Barber MRW, Drenkard C, Falasinnu T, Hoi A, Mak A, Kow NY, et al. Global epidemiology of systemic lupus erythematosus. Nat Rev Rheumatol [Internet]. 2021;17(9):515–32. Available from: https://doi.org/10.1038/s41584-021-00668-1

22. Mok CC. Current role of rituximab in systemic lupus erythematosus. Int J Rheum Dis. 2015 Feb;18(2):154–63.

23. Bednarski JJ, Sleckman BP. At the intersection of DNA damage and immune responses. Nat Rev Immunol [Internet]. 2019;19(4):231–42. Available from: http://dx.doi.org/10.1038/s41577-019-0135-6

24. Heylmann D, Rödel F, Kindler T, Kaina B. Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. Biochim Biophys Acta. 2014 Aug;1846(1):121–9.

25. Heylmann D, Ponath V, Kindler T, Kaina B. Comparison of DNA repair and radiosensitivity of different blood cell populations. Sci Rep [Internet]. 2021;11(1):1–13. Available from: https://doi.org/10.1038/s41598-021-81058-1

26. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature [Internet]. 2010;461(7267):1071–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2906700&tool=pmcentrez&rendertype=abstract

27. Giglia-Mari G, Zotter A, Vermeulen W. DNA damage response. Cold Spring Harb Perspect Biol. 2011;3(1):1–19.

28. Stratigopoulou M, van Dam TP, Guikema JEJ. Base Excision Repair in the Immune System: Small DNA Lesions With Big Consequences. Front Immunol. 2020;11(May).

29. Vilenchik MM, Knudson AG. Endogenous DNA double-strand breaks: Production, fidelity of repair, and induction of cancer. Proc Natl Acad Sci U S A. 2003;100(22):12871–6.

30. Lindahl T. Instability and decay of the primary structure of DNA. Nature. 1993;362(6422):709–15.

31. Manolakou T, Verginis P, Boumpas DT. DNA Damage Response in the Adaptive Arm of the Immune System: Implications for Autoimmunity. Int J Mol Sci. 2021 May;22(11).

32. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen. 2017 Jun;58(5):235–63.

33. Zhou BS, Elledge SJ. Checkpoints in Perspective. Nature. 2000;408(November):433–9.

34. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev. 2001;15(17):2177–96.

35. Sancar A, Lindsey-Boltz LA, Ünsal-Kaçmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem. 2004;73:39–85.

36. Zhou BBS, Bartek J. Targeting the checkpoint kinases: Chemosensitization versus chemoprotection. Nat Rev Cancer. 2004;4(3):216–25.

37. Agami R, Bernards R. Distinct Initiation and Maintenance Mechanisms Cooperate to Induce G1 Cell Cycle Arrest in Response to DNA Damage CDK2 and CDK4 complexes, but act as potent inhibitors of cyclin E-CDK2 protein complexes and as positive regulators in the case of D cyclins-. Cell. 2000;102:55–66.

38. Nakad R, Schumacher B. DNA damage response and immune defense: Links and mechanisms. Front Genet. 2016;7(AUG):1–10.

39. Rodier F, Coppé JP, Patil CK, Hoeijmakers WAM, Muñoz DP, Raza SR, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol. 2009;11(8):973–9.

40. Neves-Costa A, Moita LF. Modulation of inflammation and disease tolerance by DNA damage response pathways. FEBS J. 2017;284(5):680–98.

41. Xu Y. DNA damage: A trigger of innate immunity but a requirement for adaptive immune homeostasis. Nat Rev Immunol. 2006;6(4):261–70.

42. Pateras IS, Havaki S, Nikitopoulou X, Vougas K, Townsend PA, Panayiotidis MI, et al. The DNA damage response and immune signaling alliance: Is it good or bad? Nature decides when and where. Pharmacol Ther [Internet]. 2015;154:36–56. Available from: http://dx.doi.org/10.1016/j.pharmthera.2015.06.011

43. Ribezzo F, Shiloh Y, Schumacher B. Systemic DNA damage responses in aging and diseases. Semin Cancer Biol. 2016;37–38:26–35.

44. Moretton A, Loizou JI. Interplay between cellular metabolism and the dna damage response in cancer. Cancers (Basel). 2020;12(8):1–29.

45. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science (80-). 2008;319(5868):1352–5.

46. Wang XS, Lee BJ, Zha S. The recent advances in non-homologous end-joining through

the lens of lymphocyte development. DNA Repair (Amst) [Internet]. 2020;94:102874. Available from: https://www.sciencedirect.com/science/article/pii/S1568786420301221

47. Methot SP, Di Noia JM. Chapter Two - Molecular Mechanisms of Somatic Hypermutation and Class Switch Recombination. In: Alt FW, editor. Academic Press; 2017. p. 37–87. (Advances in Immunology; vol. 133). Available from: https://www.sciencedirect.com/science/article/pii/S0065277616300530

48. Hoeijmakers JHJ. DNA Damage, Aging, and Cancer. N Engl J Med. 2009;361(15):1475–85.

49. Ragu S, Matos-Rodrigues G, Lopez BS. Replication stress, DNA damage, inflammatory cytokines and innate immune response. Genes (Basel). 2020;11(4).

50. Chatzinikolaou G, Karakasilioti I, Garinis GA. DNA damage and innate immunity: links and trade-offs. Trends Immunol [Internet]. 2014 Sep 1;35(9):429–35. Available from: https://doi.org/10.1016/j.it.2014.06.003

51. Janeway CA Jr, Travers P, Walport M et al. Immunobiology: The Immune System in Health and Disease. [Internet]. 5th editio. New York: Garland Science; 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27155/

52. Bashir S, Harris G, Denman MA, Blake DR, Winyard PG. Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. Ann Rheum Dis. 1993;52(9):659–66.

53. Diamond B, Scharff MD. Somatic mutation of the T15 heavy chain gives rise to an antibody with autoantibody specificity. Proc Natl Acad Sci U S A. 1984;81(18 I):5841–4.

54. Harris G, Lawley PD, Asbery L, Denman AM, Hylton W. DEFECTIVE REPAIR OF 0^6-METHYLGUANINE IN AUTOIMMUNE DISEASES. Lancet [Internet]. 1982 Oct 30;320(8305):952–6. Available from: https://doi.org/10.1016/S0140-6736(82)90159-3

55. Harris G, Cramp WA, Edwards JC, George AM, Sabovljev SA, Hart L, et al. Radiosensitivity of peripheral blood lymphocytes in autoimmune disease. Int J Radiat Biol. 1985;47(6):689–99.

56. Yang YG, Lindahl T, Barnes DE. Trex1 Exonuclease Degrades ssDNA to Prevent Chronic Checkpoint Activation and Autoimmune Disease. Cell. 2007;131(5):873–86.

57. Rice G, Newman WG, Dean J, Patrick T, Parmar R, Flintoff K, et al. Heterozygous mutations in TREX1 cause familial chilblain lupus and dominant Aicardi-Goutières syndrome. Am J Hum Genet. 2007;80(4):811–5.

58. Rice G, Patrick T, Parmar R, Taylor CF, Aeby A, Aicardi J, et al. Clinical and molecular phenotype of Aicardi-Goutières syndrome. Am J Hum Genet. 2007;81(4):713–25.

59. Richards A, Van Den Maagdenberg AMJM, Jen JC, Kavanagh D, Bertram P, Spitzer D, et al. C-terminal truncations in human 3'-5' DNA exonuclease TREX1 cause autosomal dominant retinal vasculopathy with cerebral leukodystrophy. Nat Genet. 2007;39(9):1068–70.

60. Gall A, Treuting P, Elkon KB, Loo Y-M, Gale Jr M, Barber GN, et al. Autoimmunity initiates in nonhematopoietic cells and progresses via lymphocytes in an interferon-dependent autoimmune disease. Immunity [Internet]. 2012 Jan 27;36(1):120–31. Available from: https://pubmed.ncbi.nlm.nih.gov/22284419

61. Cazzalini O, Scovassi AI, Savio M, Stivala LA, Prosperi E. Multiple roles of the cell cycle inhibitor p21CDKN1A in the DNA damage response. Mutat Res Mutat Res [Internet]. 2010;704(1):12–20. Available from: https://www.sciencedirect.com/science/article/pii/S138357421000013X

62. Dutto I, Sukhanova M, Tillhon M, Cazzalini O, Stivala LA, Scovassi AI, et al. p21CDKN1A Regulates the Binding of Poly(ADP-Ribose) Polymerase-1 to DNA Repair Intermediates. PLoS One. 2016;11(1):e0146031.

63. Daszkiewicz L, Vázquez-Mateo C, Rackov G, Ballesteros-Tato A, Weber K, Madrigal-Avilés A, et al. Distinct p21 requirements for regulating normal and self-reactive T cells through IFN-γ production. Sci Rep [Internet]. 2015;5(1):7691. Available from: https://doi.org/10.1038/srep07691

64. Balomenos D, Martín-Caballero J, García MI, Prieto I, Flores JM, Serrano M, et al. The cell cycle inhibitor p21 controls T-cell proliferation and sex-linked lupus development. Nat Med. 2000 Feb;6(2):171–6.

65. Lawson BR, Baccala R, Song J, Croft M, Kono DH, Theofilopoulos AN. Deficiency of the cyclin kinase inhibitor p21(WAF-1/CIP-1) promotes apoptosis of activated/memory T cells and inhibits spontaneous systemic autoimmunity. J Exp Med. 2004 Feb;199(4):547–57.

66. Senejani AG, Liu Y, Kidane D, Maher SE, Zeiss CJ, Park HJ, et al. Mutation of POLB causes lupus in mice. Cell Rep [Internet]. 2014;6(1):1–8. Available from: http://dx.doi.org/10.1016/j.celrep.2013.12.017

67. Herkel J, Mimran A, Erez N, Kam N, Lohse AW, Märker-Hermann E, et al. Autoimmunity to the p53 protein is a feature of systemic lupus erythematosus (SLE) related to anti-DNA antibodies. J Autoimmun. 2001;17(1):63–9.

68. Luo H, Wang L, Bao D, Wang L, Zhao H, Lian Y, et al. Novel Autoantibodies Related to Cell Death and DNA Repair Pathways in Systemic Lupus Erythematosus. Genomics, Proteomics Bioinforma [Internet]. 2019;17(3):248–59. Available from: https://doi.org/10.1016/j.gpb.2018.11.004

69. Shao L, Fujii H, Colmegna I, Oishi H, Goronzy JJ, Weyand CM. Deficiency of the DNA repair enzyme ATM in rheumatoid arthritis. J Exp Med. 2009;206(6):1435–49.

70. Schild-Poulter C, Su A, Shih A, Kelly OP, Fritzler MJ, Goldstein R, et al. Association of autoantibodies with Ku and DNA repair proteins in connective tissue diseases. Rheumatology. 2008;47(2):165–71.

71. McNally JP, Millen SH, Chaturvedi V, Lakes N, Terrell CE, Elfers EE, et al. Manipulating DNA damage-response signaling for the treatment of immune-mediated diseases. Proc Natl Acad Sci U S A. 2017;114(24):E4782–91.

72. Hu Q, Xie Y, Ge Y, Nie X, Tao J, Zhao Y. Resting T cells are hypersensitive to DNA damage due to defective DNA repair pathway. Cell Death Dis [Internet]. 2018;9(6). Available from: http://dx.doi.org/10.1038/s41419-018-0649-z

73. Namas R, Renauer P, Ognenovski M, Tsou P-S, Sawalha AH. Histone H2AX phosphorylation as a measure of DNA double-strand breaks and a marker of environmental stress and disease activity in lupus. Lupus Sci Med. 2016;3(1):e000148.

74. Li Y, Zhao M, Yin H, Gao F, Wu X, Luo Y, et al. Overexpression of the growth arrest and DNA damage-induced 45α gene contributes to autoimmunity by promoting DNA demethylation in lupus T cells. Arthritis Rheum. 2010;62(5):1438–47.

75. Salvador JM, Hollander MC, Nguyen AT, Kopp JB, Barisoni L, Moore JK, et al. Mice lacking the p53-effector gene Gadd45a develop a lupus-like syndrome. Immunity. 2002 Apr;16(4):499–508.

76. Yang Z, Shen Y, Oishi H, Matteson EL, Tian L, Goronzy JJ, et al. Restoring oxidant signaling suppresses proarthritogenic T cell effector functions in rheumatoid arthritis. Sci Transl Med. 2016 Mar;8(331):331ra38.

77. Alissafi T, Kalafati L, Lazari M, Filia A, Kloukina I, Manifava M, et al. Mitochondrial Oxidative Damage Underlies Regulatory T Cell Defects in Autoimmunity. Cell Metab [Internet]. 2020;32(4):591-604.e7. Available from: https://doi.org/10.1016/j.cmet.2020.07.001

78. Lai FPL, Tsukada Y, Ichikawa H, Dunster K, Sentry JW, Toh BH. Autoantibody to DNA excision repair enzyme hMYH in a patient with rheumatic disease. Cell Immunol. 2001 Mar 15;208(2):291–7.

79. Casciola-Rosen LA, Pluta AF, Plotz PH, Cox AE, Morris S, Wigley FM, et al. The DNA mismatch repair enzyme PMS1 is a myositis-specific autoantigen. Arthritis Rheum. 2001;44(2):389–96.

80. Noble PW, Bernatsky S, Clarke AE, Isenberg DA, Ramsey-Goldman R, Hansen JE. DNA-damaging autoantibodies and cancer: The lupus butterfly theory. Nat Rev Rheumatol [Internet]. 2016;12(7):429–34. Available from: http://dx.doi.org/10.1038/nrrheum.2016.23

81. Sheng Y-J, Gao J-P, Li J, Han J-W, Xu Q, Hu W-L, et al. Follow-up study identifies two novel susceptibility loci PRKCB and 8p11.21 for systemic lupus erythematosus. Rheumatology (Oxford). 2011 Apr;50(4):682–8.

82. Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, et al. Genetics and beyond--the transcriptome of human monocytes and disease susceptibility. PLoS One. 2010 May;5(5):e10693.

83. Mensah KA, Chen JW, Schickel JN, Isnardi I, Yamakawa N, Vega-Loza A, et al. Impaired ATM activation in B cells is associated with bone resorption in rheumatoid arthritis. Sci Transl Med. 2019;11(519).

84. Erttmann SF, Härtlova A, Sloniecka M, Raffi FAM, Hosseinzadeh A, Edgren T, et al. Loss of the DNA Damage Repair Kinase ATM Impairs Inflammasome-Dependent Anti-Bacterial Innate Immunity. Immunity. 2016;45(1):106–18.

85. Davies RC, Pettijohn K, Fike F, Wang J, Nahas SA, Tunuguntla R, et al. Defective DNA double-strand break repair in pediatric systemic lupus erythematosus. Arthritis Rheum. 2012;64(2):568–78.

86. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel A V, Wang X, et al. Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. Immunity. 2018 Oct;49(4):725-739.e6.

87. Licandro G, Ling Khor H, Beretta O, Lai J, Derks H, Laudisi F, et al. The NLRP3 inflammasome affects DNA damage responses after oxidative and genotoxic stress in dendritic cells. Eur J Immunol. 2013;43(8):2126–37.

88. Lu A, Li H, Niu J, Wu S, Xue G, Yao X, et al. Hyperactivation of the NLRP3 Inflammasome in Myeloid Cells Leads to Severe Organ Damage in Experimental Lupus. J Immunol. 2017;198(3):1119–29.

89. Li Z, Guo J, Bi L. Role of the NLRP3 inflammasome in autoimmune diseases. Biomed Pharmacother [Internet]. 2020;130:110542. Available from: https://www.sciencedirect.com/science/article/pii/S0753332220307356

90. So EY, Ouchi T. Translational initiation regulated by ATM in dendritic cells development. Cell Death Dis [Internet]. 2014;5(9):e1418–e1418. Available from: https://doi.org/10.1038/cddis.2014.362

91. Petersone L, Edner NM, Ovcinnikovs V, Heuts F, Ross EM, Ntavli E, et al. T Cell/B Cell Collaboration and Autoimmunity: An Intimate Relationship. Front Immunol. 2018;9(August):1941.

92. Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature. 2017;542(7639):110–4.

93. Mitkin NA, Muratova AM, Sharonov G V., Korneev K V., Sviriaeva EN, Mazurov D, et al. p63 and p73 repress CXCR5 chemokine receptor gene expression in p53-deficient MCF-7 breast cancer cells during genotoxic stress. Biochim Biophys Acta - Gene Regul Mech [Internet]. 2017;1860(12):1169–78. Available from: http://dx.doi.org/10.1016/j.bbagrm.2017.10.003

94. Shao L. Dna damage response signals transduce stress from rheumatoid arthritis risk factors into t cell dysfunction. Front Immunol. 2018;9(December):1–8.

95. Gianchecchi E, Delfino DV, Fierabracci A. NK cells in autoimmune diseases: Linking innate and adaptive immune responses. Autoimmun Rev [Internet]. 2018;17(2):142–54. Available from: http://dx.doi.org/10.1016/j.autrev.2017.11.018

96. Mak TW, Saunders ME, Jett BDBT-P to the IR (Second E, editors. Chapter 11 - NK, γδ T and NKT Cells. In Boston: Academic Cell; 2014. p. 247–68. Available from: https://www.sciencedirect.com/science/article/pii/B978012385245800011X

97. Wu L, Kaer L. Natural Killer T Cells and Autoimmune Disease. Curr Mol Med. 2009;9(1):4–14.

98. Johansson S, Berg L, Hall H, Höglund P. NK cells: Elusive players in autoimmunity. Trends Immunol. 2005;26(11):613–8.

99. Lünemann A, Lünemann JD, Münz C. Regulatory NK-cell functions in inflammation and autoimmunity. Mol Med. 2009;15(9–10):352–8.

100. Karo JM, Schatz DG, Sun JC. The RAG recombinase dictates functional heterogeneity and cellular fitness in natural killer cells. Cell. 2014 Sep;159(1):94–107.

101. Fugmann SD, Lee AI, Shockett PE, Villey IJ, Schatz DG. The RAG proteins and V(D)J recombination: complexes, ends, and transposition. Annu Rev Immunol. 2000;18:495–527.

102. Schlissel MS. Regulating antigen-receptor gene assembly. Nat Rev Immunol [Internet]. 2003;3(11):890–9. Available from: https://doi.org/10.1038/nri1225

103. Geier CB, Farmer JR, Foldvari Z, Ujhazi B, Steininger J, Sleasman JW, et al. Vasculitis as a Major Morbidity Factor in Patients With Partial RAG Deficiency. Front Immunol. 2020;11(October).

104. Chen K, Wu W, Mathew D, Zhang Y, Browne SK, Rosen LB, et al. Autoimmunity due to RAG deficiency and estimated disease incidence in RAG1/2 mutations. Vol. 133, The Journal of allergy and clinical immunology. 2014. p. 880-2.e10.

105. Soriani A, Zingoni A, Cerboni C, Iannitto ML, Ricciardi MR, Di Gialleonardo V, et al. ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. Blood. 2009 Apr;113(15):3503–11.

106. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature. 2005;436(7054):1186–90.

107. Groh V, Brühl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. Proc Natl Acad Sci U S A. 2003;100(16):9452–7.

108. Andersson AK, Sumariwalla PF, McCann FE, Amjadi P, Chang C, McNamee K, et al. Blockade of NKG2D ameliorates disease in mice with collagen-induced arthritis: A potential pathogenic role in chronic inflammatory arthritis. Arthritis Rheum. 2011;63(9):2617–29.

109. Hüe S, Mention JJ, Monteiro RC, Zhang SL, Cellier C, Schmitz J, et al. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. Immunity. 2004;21(3):367–77.

110. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, et al. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 2004;21(3):357–66.

111. Shi F-D, Takeda K, Akira S, Sarvetnick N, Ljunggren H-G. IL-18 Directs Autoreactive T Cells and Promotes Autodestruction in the Central Nervous System Via Induction of IFN-γ by NK Cells. J Immunol. 2000;165(6):3099–104.

112. Van Belle TL, von Herrath MG. The role of the activating receptor NKG2D in autoimmunity. Mol Immunol. 2009;47(1):8–11.

113. Ogasawara K, Hamerman JA, Ehrlich LR, Bour-Jordan H, Santamaria P, Bluestone JA, et al. NKG2D blockade prevents autoimmune diabetes in NOD mice. Immunity. 2004 Jun;20(6):757–67.

114. Swann JB, Uldrich AP, Van Dommelen S, Sharkey J, Murray WK, Godfrey DI, et al. Type I natural killer T cells suppress tumors caused by p53 loss in mice. Blood. 2009;113(25):6382–5.

115. Shabrish S, Mittra I. Cytokine Storm as a Cellular Response to dsDNA Breaks: A New Proposal. Front Immunol. 2021;12(February):1–10.

116. Mittra I, Samant U, Sharma S, Raghuram G V, Saha T, Tidke P, et al. Cell-free chromatin from dying cancer cells integrate into genomes of bystander healthy cells to induce DNA damage and inflammation. Cell Death Discov [Internet]. 2017;3(1):17015. Available from: https://doi.org/10.1038/cddiscovery.2017.15

117. Würtele H, Little KCE, Chartrand P. Illegitimate DNA integration in mammalian cells. Gene Ther [Internet]. 2003;10(21):1791–9. Available from: https://doi.org/10.1038/sj.gt.3302074

118. Duvvuri B, Lood C. Cell-Free DNA as a Biomarker in Autoimmune Rheumatic Diseases. Front Immunol [Internet]. 2019;10:502. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2019.00502

119. Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol. 1966

Mar;96(3):464–71.

120. Liu X, Fang L, Guo TB, Mei H, Zhang JZ. Drug targets in the cytokine universe for autoimmune disease. Trends Immunol [Internet]. 2013;34(3):120–8. Available from: https://www.sciencedirect.com/science/article/pii/S1471490612001780

121. Bird L. Targeting cytokines in disease. Nat Immunol [Internet]. 2016;17(1):S17–S17. Available from: https://doi.org/10.1038/ni.3612

122. Gunderson CC, Moore KN. Olaparib: an oral PARP-1 and PARP-2 inhibitor with promising activity in ovarian cancer. Future Oncol. 2015;11(5):747–57.

123. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005 Apr;434(7035):913–7.

124. Pearl LH, Schierz AC, Ward SE, Al-Lazikani B, Pearl FMG. Therapeutic opportunities within the DNA damage response. Nat Rev Cancer [Internet]. 2015;15(3):166–80. Available from: http://dx.doi.org/10.1038/nrc3891

125. Vanpouille-Box C, Alard A, Aryankalayil MJ, Sarfraz Y, Diamond JM, Schneider RJ, et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. Nat Commun. 2017 Jun;8:15618.

126. Bernatsky S, Ramsey-Goldman R, Labrecque J, Joseph L, Boivin J-F, Petri M, et al. Cancer risk in systemic lupus: an updated international multi-centre cohort study. J Autoimmun. 2013 May;42:130–5.

127. Dey D, Kenu E, Isenberg DA. Cancer complicating systemic lupus erythematosus--a dichotomy emerging from a nested case-control study. Lupus. 2013 Aug;22(9):919–27.

128. Goobie GC, Bernatsky S, Ramsey-Goldman R, Clarke AE. Malignancies in systemic lupus erythematosus: a 2015 update. Curr Opin Rheumatol. 2015 Sep;27(5):454–60.

129. Ni J, Qiu L-J, Hu L-F, Cen H, Zhang M, Wen P-F, et al. Lung, liver, prostate, bladder malignancies risk in systemic lupus erythematosus: evidence from a meta-analysis. Lupus. 2014 Mar;23(3):284–92.

130. Hansen JE, Chan G, Liu Y, Hegan DC, Dalal S, Dray E, et al. Targeting cancer with a lupus autoantibody. Sci Transl Med. 2012 Oct;4(157):157ra142.

131. Weisbart RH, Gera JF, Chan G, Hansen JE, Li E, Cloninger C, et al. A cell-penetrating bispecific antibody for therapeutic regulation of intracellular targets. Mol Cancer Ther. 2012 Oct;11(10):2169–73.

132. Elkon R, Agami R. Characterization of noncoding regulatory DNA in the human genome. Nat Biotechnol [Internet]. 2017;35(8):732–46. Available from: https://doi.org/10.1038/nbt.3863

133. Lee H, Zhang Z, Krause HM. Long Noncoding RNAs and Repetitive Elements: Junk or Intimate Evolutionary Partners? Trends Genet. 2019 Dec;35(12):892–902.

134. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol [Internet]. 2017;18(1):83. Available from: https://doi.org/10.1186/s13059-017-1215-1

135. Ludvigsen M, Honoré B. Transcriptomics and Proteomics: Integration? [Internet]. eLS. 2018. p. 1–7. (Major Reference Works). Available from: https://doi.org/10.1002/9780470015902.a0006188.pub2

136. Panousis NI, Bertsias GK, Ongen H, Gergianaki I, Tektonidou MG, Trachana M, et al. Combined genetic and transcriptome analysis of patients with SLE: distinct, targetable signatures for susceptibility and severity. Ann Rheum Dis [Internet]. 2019 Aug 1;78(8):1079 LP – 1089. Available from: http://ard.bmj.com/content/78/8/1079.abstract

137. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Ann Rheum Dis. 2019 Sep;78(9):1151–9.

138. Wang Y, Hu Q, Madri JA, Rollins SA, Chodera A, Matis LA. Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc Natl Acad Sci U S A [Internet]. 1996 Aug 6;93(16):8563–8. Available from: https://pubmed.ncbi.nlm.nih.gov/8710910

139. Mähler Convenor M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim. 2014 Jul;48(3):178–92.

140. Hughes CS, Moggridge S, Müller T, Sorensen PH, Morin GB, Krijgsveld J. Single-pot, solid-phase-enhanced sample preparation for proteomics experiments. Nat Protoc. 2019 Jan;14(1):68–85.

141. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019 Jan;47(D1):D607–13.

142. Krämer A, Green J, Pollard JJ, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014 Feb;30(4):523–30.

143. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data. 2010; Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

144. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal; Vol 17, No 1 Next Gener Seq Data Anal - 1014806/ej171200 [Internet]. 2011 May 2; Available from: http://journal.embnet.org/index.php/embnetjournal/article/view/200

145. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013 Jan;29(1):15–21.

146. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015 Jan;31(2):166–9.

147. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010 Jan;26(1):139–40.

148. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci [Internet]. 2005;102(43):15545–50. Available from: https://www.pnas.org/content/102/43/15545

149. Canene-Adams K. Chapter Fifteen - Preparation of Formalin-fixed Paraffin-embedded Tissue for Immunohistochemistry. In: Lorsch JBT-M in E, editor. Laboratory Methods

in Enzymology: Cell, Lipid and Carbohydrate [Internet]. Academic Press; 2013. p. 225–33. Available from: https://www.sciencedirect.com/science/article/pii/B9780124200678000155

150. Alissafi T, Banos A, Boon L, Sparwasser T, Ghigo A, Wing K, et al. Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. J Clin Invest. 2017 Jun;127(7):2789–804.

151. Kolmykov S, Yevshin I, Kulyashov M, Sharipov R, Kondrakhin Y, Makeev VJ, et al. GTRD: an integrated view of transcription regulation. Nucleic Acids Res [Internet]. 2021 Jan 8;49(D1):D104–11. Available from: https://doi.org/10.1093/nar/gkaa1057

152. Praz V, Périer R, Bonnard C, Bucher P. The Eukaryotic Promoter Database, EPD: new entry types and links to gene expression data. Nucleic Acids Res. 2002 Jan;30(1):322–4.

153. Dantuma NP, van Attikum H. Spatiotemporal regulation of posttranslational modifications in the DNA damage response. EMBO J. 2016 Jan;35(1):6–23.

154. Huen MSY, Chen J. The DNA damage response pathways: at the crossroad of protein modifications. Cell Res [Internet]. 2008;18(1):8–16. Available from: https://doi.org/10.1038/cr.2007.109

155. Taurog JD, Chhabra A, Colbert RA. Ankylosing Spondylitis and Axial Spondyloarthritis. N Engl J Med [Internet]. 2016 Jun 29;374(26):2563–74. Available from: https://doi.org/10.1056/NEJMra1406182

156. Ruiz-Losada M, González R, Peropadre A, Gil-Gálvez A, Tena JJ, Baonza A, et al. Coordination between cell proliferation and apoptosis after DNA damage in Drosophila. Cell Death Differ [Internet]. 2021; Available from: https://doi.org/10.1038/s41418-021-00898-6

157. Koganti S, Hui-Yuen J, McAllister S, Gardner B, Grasser F, Palendira U, et al. STAT3 interrupts ATR-Chk1 signaling to allow oncovirus-mediated cell proliferation. Proc Natl Acad Sci U S A. 2014 Apr;111(13):4946–51.

158. Buang N, Tapeng L, Gray V, Sardini A, Whilding C, Lightstone L, et al. Type I interferons affect the metabolic fitness of CD8(+) T cells from patients with systemic lupus erythematosus. Nat Commun. 2021 Mar;12(1):1980.

159. Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. Lupus Sci & Med [Internet]. 2019 Aug 1;6(1):e000270. Available from: http://lupus.bmj.com/content/6/1/e000270.abstract

160. Cao Y, Gordic M, Kobold S, Lajmi N, Meyer S, Bartels K, et al. An optimized assay for the enumeration of antigen-specific memory B cells in different compartments of the human body. J Immunol Methods [Internet]. 2010;358(1):56–65. Available from: https://www.sciencedirect.com/science/article/pii/S0022175910000736

161. Van Belle K, Herman J, Boon L, Waer M, Sprangers B, Louat T. Comparative In Vitro Immune Stimulation Analysis of Primary Human B Cells and B Cell Lines. Tabarkiewicz J, editor. J Immunol Res [Internet]. 2016;2016:5281823. Available from: https://doi.org/10.1155/2016/5281823

162. Hawkins ED, Turner ML, Wellard CJ, Zhou JHS, Dowling MR, Hodgkin PD. Quantal and graded stimulation of B lymphocytes as alternative strategies for regulating adaptive immune responses. Nat Commun. 2013;4:2406.

163. Hasbold J, Corcoran LM, Tarlinton DM, Tangye SG, Hodgkin PD. Evidence from the

generation of immunoglobulin G–secreting cells that stochastic mechanisms regulate lymphocyte differentiation. Nat Immunol [Internet]. 2004;5(1):55–63. Available from: https://doi.org/10.1038/ni1016

164. Bankó Z, Pozsgay J, Szili D, Tóth M, Gáti T, Nagy G, et al. Induction and Differentiation of IL-10-Producing Regulatory B Cells from Healthy Blood Donors and Rheumatoid Arthritis Patients. J Immunol. 2017 Feb;198(4):1512–20.

165. Båve U, Alm G V, Rönnblom L. The Combination of Apoptotic U937 Cells and Lupus IgG Is a Potent IFN-α Inducer. J Immunol [Internet]. 2000 Sep 15;165(6):3519 LP – 3526. Available from: http://www.jimmunol.org/content/165/6/3519.abstract

166. Menon M, Blair PA, Isenberg DA, Mauri C. A Regulatory Feedback between Plasmacytoid Dendritic Cells and Regulatory B Cells Is Aberrant in Systemic Lupus Erythematosus. Immunity. 2016 Mar;44(3):683–97.

167. Middleton MR, Dean E, Evans TRJ, Shapiro GI, Pollard J, Hendriks BS, et al. Phase 1 study of the ATR inhibitor berzosertib (formerly M6620, VX-970) combined with gemcitabine ± cisplatin in patients with advanced solid tumours. Br J Cancer [Internet]. 2021;125(4):510–9. Available from: https://doi.org/10.1038/s41416-021-01405-x

168. Massey AJ. Inhibition of ATR-dependent feedback activation of Chk1 sensitises cancer cells to Chk1 inhibitor monotherapy. Cancer Lett. 2016 Dec;383(1):41–52.

169. Möckel T, Basta F, Weinmann-Menke J, Schwarting A. B cell activating factor (BAFF): Structure, functions, autoimmunity and clinical implications in Systemic Lupus Erythematosus (SLE). Autoimmun Rev. 2021 Feb;20(2):102736.

170. Vincent FB, Morand EF, Schneider P, Mackay F. The BAFF/APRIL system in SLE pathogenesis. Nat Rev Rheumatol [Internet]. 2014;10(6):365–73. Available from: https://doi.org/10.1038/nrrheum.2014.33

171. Higuchi T, Aiba Y, Nomura T, Matsuda J, Mochida K, Suzuki M, et al. Cutting Edge: Ectopic Expression of CD40 Ligand on B Cells Induces Lupus-Like Autoimmune Disease. J Immunol [Internet]. 2002;168(1):9–12. Available from: https://www.jimmunol.org/content/168/1/9

172. Desai-Mehta A, Lu L, Ramsey-Goldman R, Datta SK. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. J Clin Invest. 1996 May;97(9):2063–73.

173. Koshy M, Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. J Clin Invest. 1996 Aug;98(3):826–37.

174. Bacalao MA, Satterthwaite AB. Recent Advances in Lupus B Cell Biology: PI3K, IFNγ, and Chromatin [Internet]. Vol. 11, Frontiers in Immunology . 2021. p. 3429. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2020.615673

175. Liu Z, Zeng W, Huang X, Wang S, Zheng J, Pan M, et al. Peripheral CD19hi B cells exhibit activated phenotype and functionality in promoting IgG and IgM production in human autoimmune diseases. Sci Rep [Internet]. 2017;7(1):13921. Available from: https://doi.org/10.1038/s41598-017-14089-2

176. Menard LC, Habte S, Gonsiorek W, Lee D, Banas D, Holloway DA, et al. B cells from African American lupus patients exhibit an activated phenotype. JCI insight. 2016 Jun;1(9):e87310.

177. Han S, Zhuang H, Shumyak S, Yang L, Reeves WH. Mechanisms of autoantibody

production in systemic lupus erythematosus. Front Immunol. 2015;6:228.

178. McConnell JR, Crockard AD, Cairns AP, Bell AL. Neutrophils from systemic lupus erythematosus patients demonstrate increased nuclear DNA damage. Clin Exp Rheumatol. 2002;20(5):653–60.

179. Namas R, Renauer P, Ognenovski M, Tsou PS, Sawalha AH. Histone H2AX phosphorylation as a measure of DNA double-strand breaks and a marker of environmental stress and disease activity in lupus. Lupus Sci Med. 2016;3(1):1–6.

180. Souliotis VL, Sfikakis PP. Increased DNA double-strand breaks and enhanced apoptosis in patients with lupus nephritis. Lupus. 2015 Jul;24(8):804–15.

181. Taher TE, Parikh K, Flores-Borja F, Mletzko S, Isenberg DA, Peppelenbosch MP, et al. Protein phosphorylation and kinome profiling reveal altered regulation of multiple signaling pathways in B lymphocytes from patients with systemic lupus erythematosus. Arthritis Rheum. 2010 Aug;62(8):2412–23.

182. Derenzini E, Agostinelli C, Imbrogno E, Iacobucci I, Casadei B, Brighenti E, et al. Constitutive activation of the DNA damage response pathway as a novel therapeutic target in diffuse large B-cell lymphoma. Oncotarget. 2015 Mar;6(9):6553–69.

183. Rossi A, Orecchioni S, Falvo P, Tabanelli V, Baiardi E, Agostinelli C, et al. Dual targeting of the DNA damage response pathway and BCL-2 in diffuse large B-cell lymphoma. Leukemia. 2021 Jul;

184. Ranuncolo SM, Polo JM, Dierov J, Singer M, Kuo T, Greally J, et al. Bcl-6 mediates the germinal center B cell phenotype and lymphomagenesis through transcriptional repression of the DNA-damage sensor ATR. Nat Immunol. 2007 Jul;8(7):705–14.

185. Restelli V, Lupi M, Chilà R, Vagni M, Tarantelli C, Spriano F, et al. DNA Damage Response Inhibitor Combinations Exert Synergistic Antitumor Activity in Aggressive B-Cell Lymphomas. Mol Cancer Ther. 2019 Jul;18(7):1255–64.

186. Mordasini V, Ueda S, Aslandogmus R, Berger C, Gysin C, Hühn D, et al. Activation of ATR-Chk1 pathway facilitates EBV-mediated transformation of primary tonsillar B-cells. Oncotarget. 2017 Jan;8(4):6461–74.

187. Schoeler K, Jakic B, Heppke J, Soratroi C, Aufschnaiter A, Hermann-Kleiter N, et al. CHK1 dosage in germinal center B cells controls humoral immunity. Cell Death Differ. 2019 Dec;26(12):2551–67.

188. Schuler F, Weiss JG, Lindner SE, Lohmüller M, Herzog S, Spiegl SF, et al. Checkpoint kinase 1 is essential for normal B cell development and lymphomagenesis. Nat Commun. 2017 Nov;8(1):1697.

189. Sun X, Liu M, Bai J, Xu J, Zhu C, Dong J, et al. ATR kinase activity promotes antibody class switch recombination in B cells through cell cycle regulation without suppressing DSB resection and microhomology usage. J Leukoc Biol. 2021 Dec;110(6):1101–12.

190. Bernatsky S, Ramsey-Goldman R, Rajan R, Boivin J-F, Joseph L, Lachance S, et al. Non-Hodgkin's lymphoma in systemic lupus erythematosus. Ann Rheum Dis. 2005 Oct;64(10):1507–9.

191. Yu Q, Katlinskaya Y V., Carbone CJ, Zhao B, Katlinski K V., Zheng H, et al. DNA-Damage-Induced Type I Interferon Promotes Senescence and Inhibits Stem Cell Function. Cell Rep [Internet]. 2015;11(5):785–97. Available from: http://dx.doi.org/10.1016/j.celrep.2015.03.069

192. Morales AJ, Carrero JA, Hung PJ, Tubbs AT, Andrews JM, Edelson BT, et al. A type I IFN-dependent DNA damage response regulates the genetic program and inflammasome activation in macrophages. Elife. 2017 Mar;6.

193. Deeks ED. Anifrolumab: First Approval. Drugs. 2021 Oct;81(15):1795–802.

194. Furlan A, Forner G, Cipriani L, Vian E, Rigoli R, Gherlinzoni F, et al. COVID-19 in B Cell-Depleted Patients After Rituximab: A Diagnostic and Therapeutic Challenge. Front Immunol [Internet]. 2021;12. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2021.763412

195. Cauwels A, Van Lint S, Paul F, Garcin G, De Koker S, Van Parys A, et al. Delivering Type I Interferon to Dendritic Cells Empowers Tumor Eradication and Immune Combination Treatments. Cancer Res. 2018 Jan;78(2):463–74.

196. Cauwels A, Van Lint S, Rogge E, Verhee A, Van Den Eeckhout B, Pang S, et al. Targeting IFN activity to both B cells and plasmacytoid dendritic cells induces a robust tolerogenic response and protection against EAE. Sci Rep. 2021 Nov;11(1):21575.

197. Schmid D, Park CG, Hartl CA, Subedi N, Cartwright AN, Puerto RB, et al. T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. Nat Commun. 2017 Nov;8(1):1747.

198. Kowalczyk-Quintas C, Chevalley D, Willen L, Jandus C, Vigolo M, Schneider P. Inhibition of Membrane-Bound BAFF by the Anti-BAFF Antibody Belimumab. Front Immunol. 2018;9:2698.

199. Nicoletti AM, Kenny CH, Khalil AM, Pan Q, Ralph KLM, Ritchie J, et al. Unexpected Potency Differences between B-Cell-Activating Factor (BAFF) Antagonist Antibodies against Various Forms of BAFF: Trimer, 60-Mer, and Membrane-Bound. J Pharmacol Exp Ther. 2016 Oct;359(1):37–44.

200. Buisson R, Boisvert JL, Benes CH, Zou L. Distinct but Concerted Roles of ATR, DNA-PK, and Chk1 in Countering Replication Stress during S Phase. Mol Cell [Internet]. 2015/09/10. 2015 Sep 17;59(6):1011–24. Available from: https://pubmed.ncbi.nlm.nih.gov/26365377

201. Toledo LI, Murga M, Zur R, Soria R, Rodriguez A, Martinez S, et al. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. Nat Struct Mol Biol [Internet]. 2011/05/08. 2011 Jun;18(6):721–7. Available from: https://pubmed.ncbi.nlm.nih.gov/21552262

202. Luciani MG, Oehlmann M, Blow JJ. Characterization of a novel ATR-dependent, Chk1-independent, intra-S-phase checkpoint that suppresses initiation of replication in Xenopus. J Cell Sci [Internet]. 2004;117(25):6019–30. Available from: https://doi.org/10.1242/jcs.01400

203. Menolfi D, Jiang W, Lee BJ, Moiseeva T, Shao Z, Estes V, et al. Kinase-dead ATR differs from ATR loss by limiting the dynamic exchange of ATR and RPA. Nat Commun [Internet]. 2018;9(1):5351. Available from: https://doi.org/10.1038/s41467-018-07798-3

204. Schuler F, Weiss JG, Lindner SE, Lohmüller M, Herzog S, Spiegl SF, et al. Checkpoint kinase 1 is essential for normal B cell development and lymphomagenesis. Nat Commun [Internet]. 2017;8(1):1–12. Available from: http://dx.doi.org/10.1038/s41467-017-01850-4

205. Speroni J, Federico MB, Mansilla SF, Soria G, Gottifredi V. Kinase-independent

function of checkpoint kinase 1 (Chk1) in the replication of damaged DNA. Proc Natl Acad Sci U S A. 2012 May;109(19):7344–9.

206. Scorah J, Dong M-Q, Yates JR 3rd, Scott M, Gillespie D, McGowan CH. A conserved proliferating cell nuclear antigen-interacting protein sequence in Chk1 is required for checkpoint function. J Biol Chem. 2008 Jun;283(25):17250–9.

207. Yang XH, Shiotani B, Classon M, Zou L. Chk1 and Claspin potentiate PCNA ubiquitination. Genes Dev. 2008 May;22(9):1147–52.

208. Maréchal A, Zou L. RPA-coated single-stranded DNA as a platform for post-translational modifications in the DNA damage response. Cell Res [Internet]. 2015;25(1):9–23. Available from: https://doi.org/10.1038/cr.2014.147

209. Jondle CN, Johnson KE, Uitenbroek AA, Sylvester PA, Nguyen C, Cui W, et al. B Cell-Intrinsic Expression of Interferon Regulatory Factor 1 Supports Chronic Murine Gammaherpesvirus 68 Infection. J Virol. 2020 Jun;94(13).

210. Uranishi M, Iida S, Sanda T, Ishida T, Tajima E, Ito M, et al. Multiple myeloma oncogene 1 (MUM1)/interferon regulatory factor 4 (IRF4) upregulates monokine induced by interferon-gamma (MIG) gene expression in B-cell malignancy. Leukemia. 2005 Aug;19(8):1471–8.

211. Mosquera Orgueira A, Ferreiro Ferro R, Díaz Arias JÁ, Aliste Santos C, Antelo Rodríguez B, Bao Pérez L, et al. Detection of new drivers of frequent B-cell lymphoid neoplasms using an integrated analysis of whole genomes. PLoS One. 2021;16(5):e0248886.

212. Mboko WP, Olteanu H, Ray A, Xin G, Darrah EJ, Kumar SN, et al. Tumor Suppressor Interferon-Regulatory Factor 1 Counteracts the Germinal Center Reaction Driven by a Cancer-Associated Gammaherpesvirus. J Virol. 2015 Dec;90(6):2818–29.

213. Scharer CD, Barwick BG, Guo M, Bally APR, Boss JM. Plasma cell differentiation is controlled by multiple cell division-coupled epigenetic programs. Nat Commun. 2018 Apr;9(1):1698.

214. Jin J, Tao Z, Cao J, Li T, Hu X. DNA damage response inhibitors: An avenue for TNBC treatment. Biochim Biophys Acta - Rev Cancer [Internet]. 2021;1875(2):188521. Available from: https://www.sciencedirect.com/science/article/pii/S0304419X21000202