The revolutionary techniques of modern molecular and cellular biology enhance almost daily our knowledge of immunity and autoimmunity in men and experimental animals. Our fragmentary puzzle of the immune system is going to form a fascinating picture of a master piece of evolution. Although many of these aspects were achieved by analysis of human body fluids and tissues, the etiopathogenesis of autoimmune diseases cannot readily be analyzed without appropriate animal models as shown in Chapter 1 and 3. Spontaneous and xenobiotic, idiotypic manipulation, and immunization induced autoimmune animal models as well as novel autoimmune knock-out and knock-in mice are used to investigate (i) the role in the pathogenesis of long-lived plasma cells, type I interferons, and mutations in genes encoding regulators of the cell cycle, (ii) the molecular mechanisms of xenobiotic autoimmunity, (iii) the break of tolerance via immunization with apoptotic material, (iv) immune mechanisms of autoimmune pregnancy loss as well as experimental APS, and (v) novel therapeutic approaches. This volume further deals with natural and pathogenic autoantibodies (Chapter 2), autoantibodies in systemic autoimmune diseases (Chapter 7), and the autoimmunity in neurological diseases. The role of B cells, autoantibodies and post-translational modifications in the pathogenesis of multiple sclerosis is discussed (Chapter 6). Some human SLE susceptibility genes identified by linkage studies e.g. at 1q23, a novel RA susceptibility gene encoding peptidylarginine deiminase type 4 as well as novel autoantibodies that target GW bodies, mitotic chromosomes, the spindle apparatus, hnRNPs, laminin-1, high mobility group box 1 protein, and the survival protein LEDGF/p75 are described in detail (Chapter 4,5). Furthermore, the occurrence and measurement of therapy-induced antibodies (Chapter 8), guidelines and approaches to autoantibody testing and new technologies in autoantibody profiling, such as addressable laser bead immunoassays and autoantigen arrays are reviewed and discussed (Chapter 9).

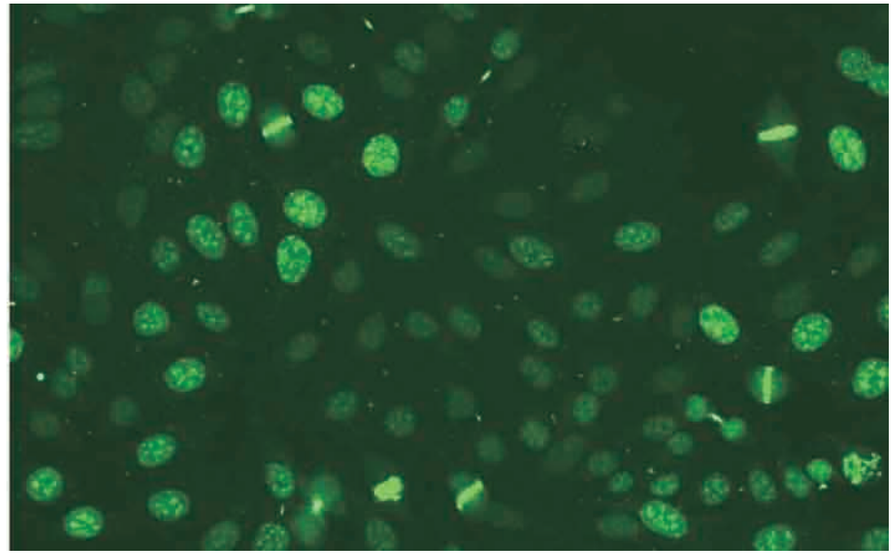
ISBN 3-89967-148-1 (Europe)
ISBN 1-59326-070-9 (USA)
www.pabst-publishers.com

From Animal Models to Human Genetics
K. Conrad, M. P. Bachmann, E. K. L. Chan, M. J. Fritzler, R. L. Humbel, U. Sack, Y. Shoenfeld (Eds.)

K. Conrad, M. P. Bachmann, E. K. L. Chan, M. J. Fritzler, R. L. Humbel, U. Sack, Y. Shoenfeld (Eds.)

From Animal Models to Human Genetics: Research on the Induction and Pathogenicity of Autoantibodies

Report on the 7th Dresden Symposium on Autoantibodies held in Dresden on September 1-4, 2004



AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY
Volume 4 - 2004

PABST

Autoantigens, autoantibodies, autoimmunity
Edited by: K. Conrad (Dresden, Germany)
U. Sack (Leipzig, Germany)
Vol. 4 – 2004

K. Conrad, M. P. Bachmann, E. K. L. Chan,
M. J. Fritzler, R. L. Humbel, U. Sack, Y. Shoenfeld
(Eds.)

From Animal Models to Human Genetics: Research on the Induction and Pathogenicity of Autoantibodies

Report on the 7th Dresden Symposium on Autoantibodies
held in Dresden on September 1-4, 2004

PABST SCIENCE PUBLISHERS
Lengerich, Berlin, Bremen, Miami,
Riga, Viernheim, Wien, Zagreb

Contents

7.4 Autoantibodies in Sjögren's Syndrome

7.5 Autoantibodies in Atherosclerosis, Recurrent Miscarriages and Antiphospholipid Syndrome

9.2 Antinuclear Antibodies

Preface

There is an ever growing number of autoimmune diseases ranging from systemic and multi-organ specific to organ-specific disorders. Worldwide, millions of people are suffering from autoimmune diseases. Many of those diseases dramatically change the life of patients and may even become life threatening. Morbidity and mortality result from the involvement of essential organs. The usual live-long treatment of autoimmune patients are very expensive and the illness is often debilitating. Unfortunately, some patients have to suffer for years until a correct diagnosis leads to appropriate or effective therapy. At least in some cases, an earlier diagnosis would improve the outcome of therapy.

What has been done, what has to be done, what can be done, now and in the future, in order to improve the diagnosis and therapy of autoimmune patients? For this purpose it is necessary to investigate the etiology and pathogenesis of the different forms of autoimmune diseases. The factors and the complex mechanisms that are involved in the development of pathological autoimmunity are incompletely understood. However, it appears to be likely that genetic as well as environmental factors are responsible for the induction, development and progression of most autoimmune diseases. Although some of the pathological aspects can be explored by analysis of human body fluids and tissues and by epidemiological studies the puzzle of the etiopathogenesis of autoimmune diseases cannot readily be analyzed without appropriate animal models. It is not astonishing therefore, that the fourth AAA volume has focused on animal autoimmune models for exploring the pathogenesis and therapy of autoimmunity in mice, the role of natural and pathogenic autoantibodies, the molecular mechanisms of xenobiotic-induced autoimmunity as well as the impact of various genes on disease development.

Another focus is on the relevance of autoantibodies in human disease as well as the genetic factors in SLE and rheumatoid arthritis (RA). The identification of PADI4, a gene encoding peptidylarginine deiminase type 4, as a RA susceptibility gene may establish ties between breaking tolerance by post-translational protein-modifications and the induction of autoantibodies to the pathogenesis and potential molecular targets for therapeutic intervention. Many open questions remain to be solved by studies on men as well as animal models.

The editors

Long-lived plasma cells contribute to humoral autoimmunity

F. Hiepe[1,2], A. Radbruch[2], R.A. Manz[2]

[1]Rheumatology and Clinical Immunology, Charité - Universitätsmedizin Berlin,
[2]German Rheumatism Research Center, Berlin, Germany

falk.hiepe@charite.de

Activation of B lymphocytes can result in their differentiation into antibody-secreting cells, plasma blasts and plasma cells. Despite their relevance for the understanding of humoral immunity, little is known about these cells which often have been considered as short-lived end stages of B cell differentiation. This view has been challenged recently. Results of several groups have shown that a substantial fraction of plasma cells can survive and continue to secrete antibody for extended periods of time in the absence of any detectable memory B cells and antigen stimulation [1-3].

Antibodies secreted by autoreactive plasma cells are a common feature of systemic and organ-specific autoimmune diseases [4-6]. They can contribute to the pathogenesis of autoimmune diseases either directly, by classic effector mechanisms, or through extensive formation and deposition of immune complexes [7]. From a clinical point of view, several hints indicate that long-lived plasma cells may play a role in autoimmune diseases. Sustained autoantibody titers can be observed in the serum of patients with autoimmune diseases following immunosuppression [8-12]. Autoantibodies directed to double-stranded DNA characterizing murine and human form of systemic lupus erythematosus (SLE) correlate with disease activity and can cause severe organ manifestations such as lupus nephritis [13-15]. They can remain stable in severe cases refractory to aggressive cyclophosphamide therapy [16,17].

We have analyzed the role of long-lived plasma cells in autoimmune-pathology in a murine model of systemic lupus erythematosus (SLE) [18]. Bromodeoxyuridine (BrdU), which is incorporated into the DNA of dividing cells, was added to the drinking water of five-month-old NZB/W mice with anti-dsDNA autoantibodies for a period of 12 weeks. We then used a plasma cell marker (CD138) in order to cytometrically distinguish between proliferating (short-lived) and non-proliferating (long-lived) cells in plasma cell compartments of the spleen (Fig. 1).

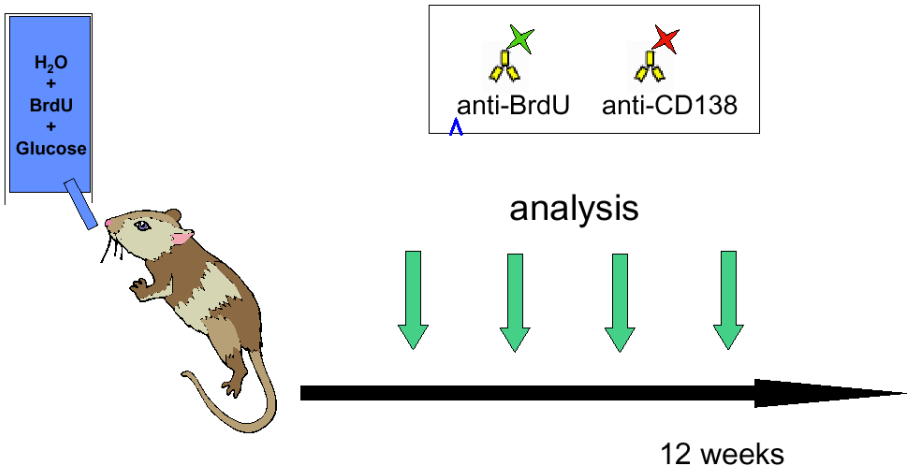


Fig. 1: Schematic depiction of analysis of long-lived plasma cells in 5 months old NZB/W mice.

About 60 % of all CD138+ cells became BrdU-positive within 10 days of BrdU feeding. The other 40 % remained unlabeled and BrdU-negative for the entire 12 weeks of BrdU feeding, indicating that these cells are long-lived, non-dividing plasma cells (Fig. 2 and 3). The total number of BrdU-negative CD138+ plasma cells did not change during these 3 months. All long-lived BrdU-negative plasma cells expressed little MHC II.

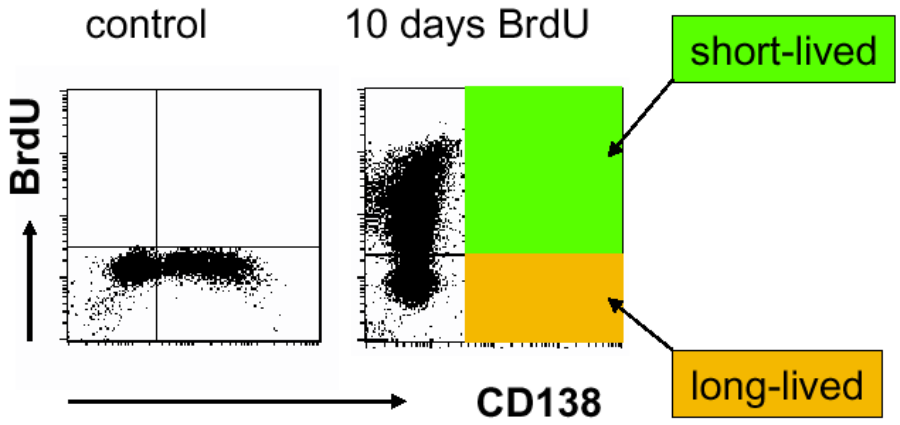


Fig. 2: Cytometric differentiation between BrdU-positive, short-lived plasma blasts and BrdU-negative, long-lived plasma cells in plasma cell compartments of the spleen of NZB/W mice after 10 days period of BrdU feeding.

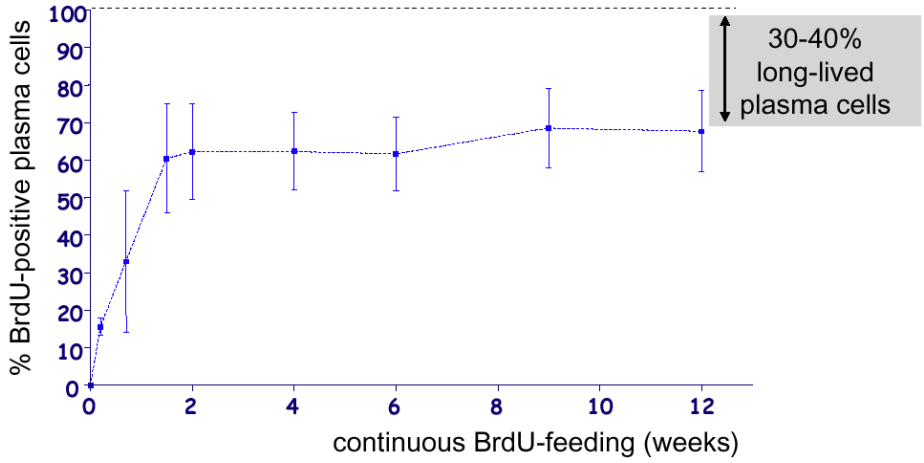


Fig. 3: Kinetics of BrdU incorporation in splenic plasma cells from NZB/W mice based on FACS analysis.

Our discovery of an unusually prominent, long-lived splenic plasma cell population in NZB/W mice made us wonder whether these cells are responsible for treatment-resistant (auto)antibody titers. In NZB/W mice treated experimentally with cyclophosphamide, the generation of short-lived plasma blasts is blocked efficiently, but long-lived plasma cells do survive (fig. 4).

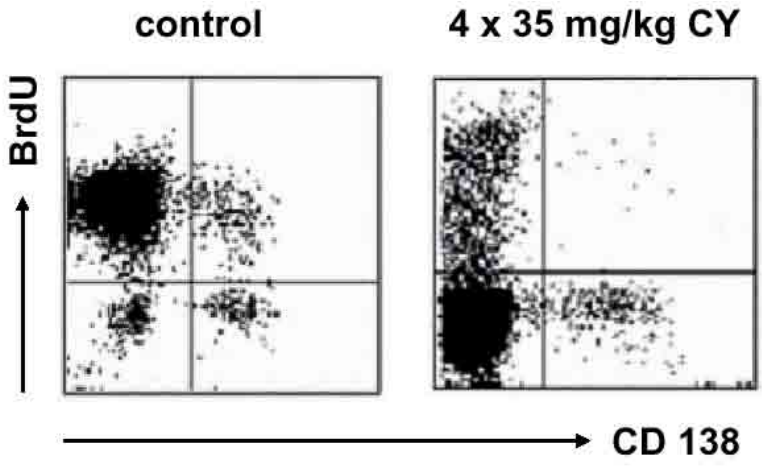


Fig. 4: Splenic BrdU-negative, long-lived plasma cells are resistant to conventional immunosuppression with high doses of cyclophosphamide whereas BrdU-negative, short-lived plasma cells do not survive this treatment. 5 months old NZB/W mice were fed BrdU for 2-4 weeks and received cyclophosphamide one week before FACS analysis.

This cyclophosphamide-resistant, long-lived plasma cell population contained a significant fraction of anti-DNA antibody-secreting cells, as identified by ELISPOT (fig. 5).

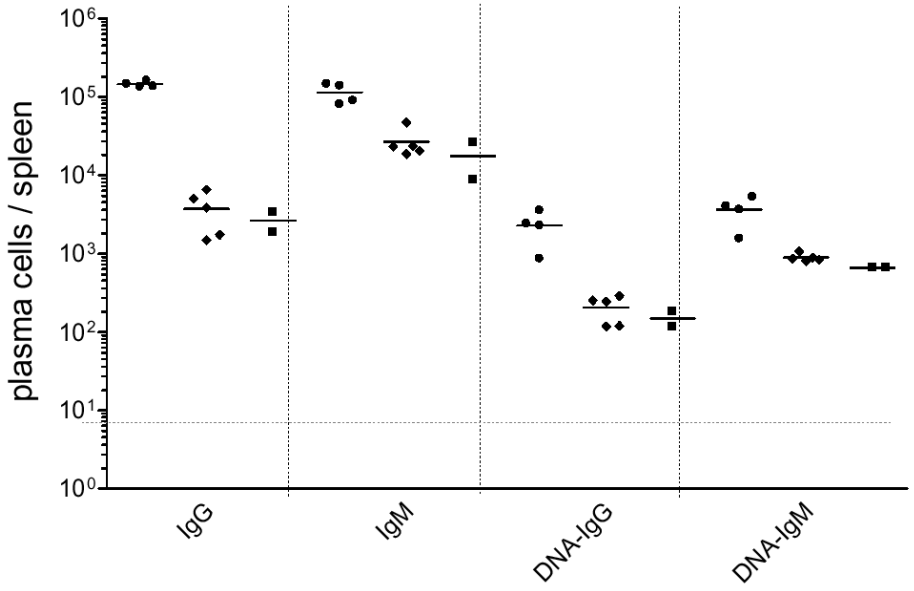


Fig. 5: ELISPOT analysis of IgG and IgM as well as IgG anti-DNA and IgM anti-DNA secreting plasma cells from the spleen of NZB/W mice shows that cyclophosphamide treatment reduces the number of Ig and anti-DNA secreting cells but there is a significant fraction of plasma cells surviving this treatment. 5 months old NZB/W mice were fed BrdU for 2-4 weeks and received cyclophosphamide one week before FACS analysis.

In NZB/W mice transgenic for the DNA-specific D42 antibody heavy chain, the frequencies of short-lived plasmablasts and long-lived plasma cells in the spleen were very similar to those observed in non-transgenic NZB/W mice, according to our BrdU incorporation data. After 12 weeks of BrdU feeding, the compartment of BrdU-negative, long-lived plasma cells contained about 20 % of D42 transgene-bearing plasma cells. This is direct evidence that autoreactive cells are contained within the compartment of long-lived plasma cells.

In these mice, both long-lived autoreactive plasma cells and short-lived, constantly *de novo* generated plasma blasts and plasma cells contribute to autoantibody levels, with a ratio of 1:3, as evidenced for dsDNA-specific antibody-secreting cells [18].

In accordance, immunosuppression of autoimmune patients by selective depletion of B lymphocytes, but not plasma cells, with anti-CD20 antibodies (Rituximab) does not lead to complete disappearance of autoantibodies [19-21]. The resistance of a subgroup of autoantibodies to Rituximab

demonstrates that these antibodies are generated by long-lived plasma cells which do not express CD20 [22]. Anti-CD20 treatment interrupts the generation of the other fraction of autoantibodies, which are secreted by short-lived plasma blasts. These cells can be visualized in peripheral blood during flares of the SLE [23-25].

Whether autoantibody-secreting cells can become long-lived plasma cells may depend on the time point and mode of their generation. The mechanisms by which the survival and migration of plasma cells are regulated are not completely understood. Recent findings suggest that plasma cell longevity is not an intrinsic capacity, but depends on a supportive environment, i.e. specific survival niches, which can be found in bone marrow, inflamed tissue and in the autoimmune spleen [26]. It has been shown that after immunization of NZB/W mice, antigen-specific antibody-secreting cells migrate not only to the bone marrow, but in similar numbers also to the chronically inflamed kidneys [27]. Very recently, autoreactive plasma cells with specificity for glomerular antigens have been demonstrated to be markedly enriched in lupus kidneys compared with the spleen and peripheral blood [28]. Anti-Ro/SSA and anti-La/SSB autoantibodies are produced and are present in lymphocyte infiltrates of inflamed salivary glands from patients with Sjögren's syndrome [29,30]. These findings suggest a potential involvement of locally produced autoantibodies in organ injury.

The fact that many more plasma cells are generated during an adaptive immune response than finally survive and become long-lived suggests that only a selected fraction of plasma cells enters the pool of long-lived plasma cells. Competition of plasma cells for survival niches thus presumably controls the establishment of humoral immunity and immunopathology [26].

It is remarkable that the spleens of NZB/W mice provide such niches for about ten times more long-lived plasma cells than those of normal mice. Factors involved in plasma cell survival are cytokines IL-5, IL-6, TNF-α, BAFF/BLys, and stromal cell-derived Factor 1α (CXCL12), as well as signaling via CD44 and BCMA expression on plasma cells. Chemokines addressing the chemokine receptors CXCR3 and CXCR4 control the migration of plasma cells, but also their survival, like CXCL12 [31-34].

Since long-lived autoreactive plasma cells are responsible for the continuous production of pathogenic autoantibodies, methods of targeting them should provide potent therapeutic approaches. Autologous stem cell transplantation (ASCT), for example, can result in the complete disappearance of all autoantibodies in patients with refractory SLE. ASCT conditioning regimens, including immunoablation by antithymocyte globulin, may therefore be effective modalities for targeting the long-lived plasma cell compartment.

References

1. Manz RA, Thiel A, Radbruch A. Lifetime of plasma cells in the bone marrow. Nature 1997; 388(6638):133-4.

2. Manz RA, Lohning M, Cassese G, Thiel A, Radbruch A. Survival of long-lived plasma cells is independent of antigen. Int Immunol 1998; 10(11):1703-11.
3. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. Immunity 1998; 8(3):363-72.
4. Davidson A, Diamond B. Autoimmune diseases. N Engl J Med 2001; 345(5):340-350.
5. Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989; 44:93-151.
6. von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 1995; 24(5):323-358.
7. Martin F, Chan AC. Pathogenic roles of B cells in human autoimmunity; insights from the clinic. Immunity 2004; 20(5):517-527.
8. De Block CE, De Leeuw IH, Rooman RP, Winnock F, Du Caju MV, Van Gaal LF. Gastric parietal cell antibodies are associated with glutamic acid decarboxylase-65 antibodies and the HLA DQA1*0501-DQB1*0301 haplotype in Type 1 diabetes mellitus. Belgian Diabetes Registry. Diabet Med 2000; 17(8):618-622.
9. Decochez K, Tits J, Coolens JL, Van Gaal L, Krzentowski G, Winnock F et al. High frequency of persisting or increasing islet-specific autoantibody levels after diagnosis of type 1 diabetes presenting before 40 years of age. The Belgian Diabetes Registry. Diabetes Care 2000; 23(6):838-844.
10. Izumi N, Fuse I, Furukawa T, Uesugi Y, Tsuchiyama J, Toba K et al. Long-term production of pre-existing alloantibodies to E and c after allogenic BMT in a patient with aplastic anemia resulting in delayed hemolytic anemia. Transfusion 2003; 43(2):241-245.
11. Wahren M, Tengner P, Gunnarsson I, Lundberg I, Hedfors E, Ringertz NR et al. Ro/SS-A and La/SS-B antibody level variation in patients with Sjogren's syndrome and systemic lupus erythematosus. J Autoimmun 1998; 11(1):29-38.
12. Westman KW, Bygren PG, Ericsson UB, Hoier-Madsen M, Wieslander J, Erfurth EM. Persistent high prevalence of thyroid antibodies after immunosuppressive therapy in subjects with glomerulonephritis. A prospective three-year follow-up study. Am J Nephrol 1998; 18(4):274-279.
13. Bruns A, Bläss S, Hausdorf G, Burmester GR, Hiepe F. Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. Arthritis Rheum 2000; 43(10):2307-2315.
14. Hahn BH. Antibodies to DNA. N Engl J Med 1998; 338(19):1359-1368.
15. Riemekasten G, Marell J, Trebeljahr G, Klein R, Hausdorf G, Haupl T et al. A novel epitope on the C-terminus of SmD1 is recognized by the majority of sera from patients with systemic lupus erythematosus. J Clin Invest 1998; 102(4):754-763.
16. Rosen O, Thiel A, Massenkeil G, Hiepe F, Häupl T, Radtke H et al. Autologous stem-cell transplantation in refractory autoimmune diseases after in vivo immunoablation and ex vivo depletion of mononuclear cells. Arthritis Res 2000; 2(4):327-336.
17. Traynor AE, Schroeder J, Rosa RM, Cheng D, Stefka J, Mujais S et al. Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study. Lancet 2000; 356(9231):701-707.
18. Hoyer BF, Moser K, Hauser AE, Peddinghaus A, Voigt C, Eilat D et al. Short-lived Plasmablasts and Long-lived Plasma Cells Contribute to Chronic Humoral Autoimmunity in NZB/W Mice. J Exp Med 2004; 199(11):1577-1584.

19. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR et al. Efficacy of B cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004; 350(25):2572-2581.
20. Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. Arthritis Rheum 2002; 46(10):2673-2677.
21. Silverman GJ, Weisman S. Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy. Arthritis Rheum 2003; 48(6):1484-1492.
22. Medina F, Segundo C, Campos-Caro A, Gonzalez-Garcia I, Brieva JA. The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. Blood 2002; 99(6):2154-61.
23. Jacobi AM, Odendahl M, Reiter K, Bruns A, Burmester GR, Radbruch A et al. Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 2003; 48(5):1332-1342.
24. Odendahl M, Jacobi A, Hansen A, Feist E, Hiepe F, Burmester GR et al. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. J Immunol 2000; 165(10):5970-9.
25. Odendahl M, Keitzer R, Wahn U, Hiepe F, Radbruch A, Dorner T et al. Perturbations of peripheral B lymphocyte homoeostasis in children with systemic lupus erythematosus. Ann Rheum Dis 2003; 62(9):851-858.
26. Manz RA, Arce S, Cassese G, Hauser AE, Hiepe F, Radbruch A. Humoral immunity and long-lived plasma cells. Curr Opin Immunol 2002; 14(4):517-21.
27. Cassese G, Lindenau S, de Boer B, Arce S, Hauser A, Riemekasten G et al. Inflamed kidneys of NZB / W mice are a major site for the homeostasis of plasma cells. Eur J Immunol 2001; 31(9):2726-32.
28. Sekine H, Watanabe H, Gilkeson GS. Enrichment of anti-glomerular antigen antibody-producing cells in the kidneys of MRL/MpJ-Fas(lpr) mice. J Immunol 2004; 172(6):3913-3921.
29. Salomonsson S, Jonsson MV, Skarstein K, Brokstad KA, Hjelmstrom P, Wahren-Herlenius M et al. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjogren's syndrome. Arthritis Rheum 2003; 48(11):3187-3201.
30. Tengner P, Halse AK, Haga HJ, Jonsson R, Wahren-Herlenius M. Detection of anti-Ro/SSA and anti-La/SSB autoantibody-producing cells in salivary glands from patients with Sjogren's syndrome. Arthritis Rheum 1998; 41(12):2238-2248.
31. Cassese G, Arce S, Hauser AE, Lehnert K, Moewes B, Mostarac M et al. Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. J Immunol 2003; 171(4):1684-90.
32. Hauser AE, Debes GF, Arce S, Cassese G, Hamann A, Radbruch A et al. Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response. J Immunol 2002; 169(3):1277-82.
33. Minges Wols HA, Underhill GH, Kansas GS, Witte PL. The role of bone marrow-derived stromal cells in the maintenance of plasma cell longevity. J Immunol 2002; 169(8):4213-21.
34. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C et al. BCMA Is Essential for the Survival of Long-lived Bone Marrow Plasma Cells. J Exp Med 2004; 199(1):91-8.

Innate immunity and interferon production in the pathogenesis of autoantibodies in lupus

D.C. Nacionales, H. Zhuang, K.M. Kelly, S. Narain, J. Weinstein, M. Honkanen-Scott, H.B. Richards, E. Sobel, M. Satoh, W.H. Reeves

Division of Rheumatology & Clinical Immunology, Center for Autoimmune Disease, University of Florida, Gainesville, FL, USA

whreeves@ufl.edu

Type I interferons (IFN)

The Type I IFNs (IFN-I), all of which have potent anti-viral activity, include 13 closely related IFN-α genes, one IFN-β and one IFN-ω gene located in the IFN gene cluster on the short arm of human chromosome 9 [1,2]. The different IFN-I proteins are expressed at different levels in virally infected cells. The expression of most IFN-α subtypes is regulated by the transcription factors IFN regulatory factor (IRF) 3 and IRF-7, whereas expression of IFN-α1 and IFN-β is regulated by IRF-3 alone [3,4]. IFN-I proteins are active as monomers and bind a specific receptor complex composed of two subunits, IFNAR1 and IFNAR2 [5]. Interactions of IFN-I with the receptor cause reciprocal transphosphorylation of the Jak non-receptor tyrosine kinases Tyk2 and Jak1, leading to receptor phosphorylation and phosphorylation of Stat1 and Stat2 (Fig. 1). IFNAR1 interacts with Tyk2 and IFNAR2 with Jak1, Stat1, and Stat2 [6]. Phosphorylation of Stat1/Stat2 leads to the activation of additional IRFs, which induce the expression of IFN regulated proteins.

Interferon inducible genes

Binding of IFN-I to the Type I IFNR increases the expression of a group of interferon stimulated genes (ISGs), which are regulated by the binding of Stat1 and/or other Stat proteins to a cis-acting consensus sequence (YAGTTTCAYTTTYCC, where Y is a pyrimidine) termed the interferon stimulated response element (ISRE). This sequence has been found in the promoter regions of all IFN-I inducible genes examined to date [7]. Transcriptional activation of IFN-I inducible genes is transient, and generally

decreases after 3-4 hours, ultimately returning to baseline. The reason for this pattern of expression is not clear, but cannot be explained entirely by down-regulation of the receptor [7]. ISGs play a key role in the anti-viral response, signal transduction, and apoptosis. Although as many as 100 genes are regulated by IFN-I [7], the subset involved in anti-viral responses is of special interest with regard to the pathogenesis of SLE. As shown in Fig. 1, activation of the Type I IFN receptor induces the expression "secondary anti-viral response genes", which include the 2'5'-oligoadenylate synthetase (OAS) family, RNAse L, dsRNA dependent protein kinase (PKR), and the GTPase Mx1 [8]. The expression of more than 20 of these genes is increased in peripheral blood mononuclear cells (PBMCs) from SLE patients [9,10]. Expression of these genes closely reflects the production of IFN-I and has been used to monitor IFN-α therapy [11]. We have used OAS, Mx1, and IRF-7 expression as a biomarker for IFN-I production.

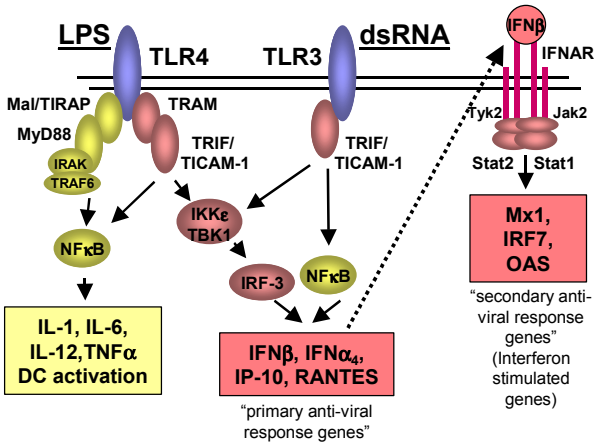


Fig. 1: MyD88 independent signalling via TLR3 and TLR4 activates Type I IFN production

Mx genes

The Mx proteins are large GTPases belonging to the dynamin family that play a key role in limiting orthomyxovirus (influenza) and rhabdovirus (vesicular stomatitis virus) infections [7,12]. In human cells, the Mx1 and Mx2 genes encode two proteins, MxA and MxB, respectively. Expression of the Mx proteins is regulated by IFN-I, but not IFN-γ. By binding to virus protein complexes, Mx protein can inhibit viral transcription and replication. Primers for SYBR green and Lux-based real-time PCR were designed for quantifying

Mx1 gene expression normalized to β-actin in murine and human cells (Fig. 2A-B, Tab. 1). These primers were shown to amplify a single product (Fig. 2C) and there was a linear relationship (r^2 = 0.971) between cycle threshold (Ct) and template concentration (Fig. 2D). We found that Mx1 gene expression is induced by IFN-I, although there was weaker induction by IFN-γ. IL-6, IL-12, and TNFα had no effect on Mx1 gene expression, suggesting that Mx1 is a suitable marker for monitoring activity of the IFN system (D.C. Nacionales et al., submitted).

Tab. 1: Primers used to quantify the expression of IFN-I inducible genes

Gene	*Species*	*Forward primer*	*Reverse primer*
Mx1	Human	ACCTCGTGTTCCACCTGAAG	GTGTGATGAGCTCGCGTGGTA
Mx1	Mouse	GATCCGACTTCACTTCCAGATGG	CATCTCAGTGGTAGTCAACCC
IRF7	Mouse	TGCTGTTTGGAGACTGGCTAT	TCCAAGCTCCCGGCTAAGT
OAS	Human	ACAGCTGAAAGCCTTTTGGA	AAGTTTCGCTGCAGGACTGT
Actin*	Human	TCCCTGGAGAAGAGCTACGA	AGCACTGTGTTGGCGTACAG
Actin*	Mouse	TGGAATCCTGTGGCATCCTGAAAC	TAAAACGCAGCTCAGTAACAGTCCG
Mx1§	Human	CACGAAGAGGCAGCGGGATCG	CCTTGCCTCTCCACTTATCTTC
Actin§*	Human	CACTGTGAAGACCTGTACGCCAACACAG	CTTCTGCATCCTGTCGGCAAT

*Control (housekeeping gene); § LUX primers (other primers are for SYBR green)

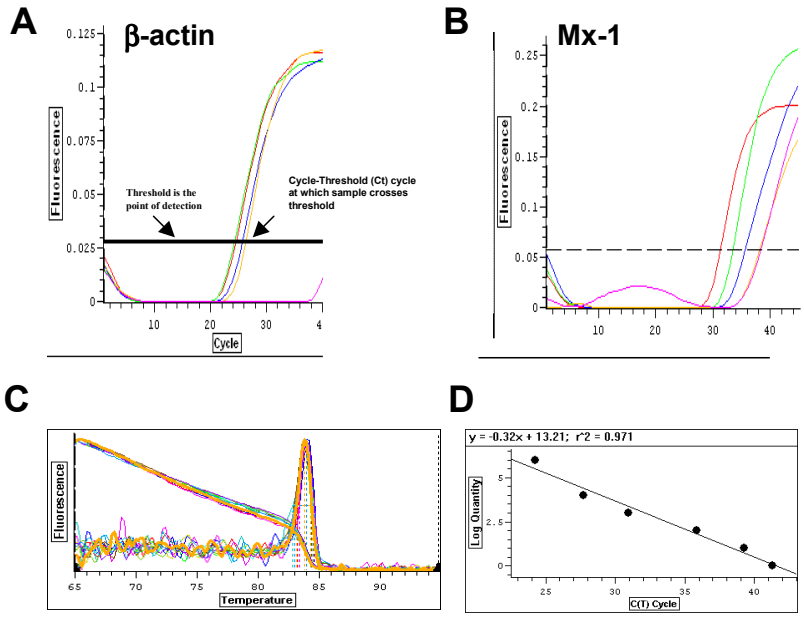


Fig. 2: Quantification of human Mx1 gene expression by real-time PCR A, Amplification curves for β-actin using mRNA from PBMCs of four patients. Note that the expression of actin is comparable in all four samples. B, Amplification curves for Mx1 for the same four patients as shown in panel A. Note that the cycle thresholds (Ct) are much more variable than in the case of actin. C, Melting curve for Mx1 indicating

that the primers amplify a single product. D, Standard curve for Mx1 showing a linear relationship between Ct and template concentration.

Interferon regulatory factor-7

As discussed above, IRF-7 is a transcription factor involved in regulating the expression of a subset of IFN-I genes that is itself IFN-regulated. PCR primers for murine IRF-7 (Tab. 1) gave results that were highly correlated with the expression of Mx-1, suggesting that the transcription of many if not most IFN regulated genes reflects IFN-I production (D.C. Nacionales et al., submitted). IRF-7 was not usable in humans, however, due to a genetic polymorphism and alternative splicing [13]. IRF-7 expression could be measured in PBMCs from a subset of healthy individuals and SLE patients, but the product could not be amplified in other individuals due to the polymorphism and the lack of other suitable primer sequences (H. Zhuang, unpublished data). Therefore, OAS gene expression was substituted for IRF-7 as a confirmatory human IFN-regulated gene.

2'5'-oligoadenylate synthetase

There are three forms of OAS, OAS1, OAS2 and OAS3, generated by differential splicing [14]. The expression of OAS is tightly regulated by IFN-I [15]. OAS catalyzes the synthesis of oligoadenylates of the structure ppp(A2'p)n (2-5A). Binding of 2-5A oligoadenylates to a latent endoribonuclease, RNAse L, causes dimerization of inactive RNase L monomers into active dimers, resulting in the degradation of both viral and cellular RNAs and cleavage of ssRNA [16]. Primers were designed to quantify human OAS gene expression by real-time PCR (Tab. 1). As in the murine system, OAS gene expression correlated highly with Mx1 expression (H. Zhuang et al., submitted). Importantly, both Mx1 and OAS gene expression increased several thousand-fold during viral upper respiratory infections, indicating that the expression of IFN-inducible genes in PBMCs can be used as a measure of IFN-I production at remote sites (H. Zhuang et al., submitted).

Cells producing Type I IFN

Although most, if not all, nucleated cells are capable of producing IFN-I, the existence of a minor population of cells in the peripheral blood that produces large amounts of IFN-I was recognized about 20 years ago [17]. These "interferon producing cells" (IPCs) have only recently been characterized [18-20]. The prototypical IPC is called a "plasmacytoid dendritic cell" (PDC) in view of its eccentrically located nucleus and prominent rough endoplasmic

reticulum (Tab. 2). In response to viral infection or oligonucleotides containing unmethylated CpG motifs, PDCs produce ~1000-fold more IFN-I than most other cell types [20]. However, it recently has become clear that myeloid dendritic cells (MDCs) also can produce large amounts of IFN-I in response to certain types of viral infections [20]. MDCs produce IFN-I following electroporation or lipofection of the double-stranded (ds) RNA analog poly (I:C) in amounts comparable to that of PDCs stimulated with CpG DNA. Thus, unlike PDCs, which are activated following endosomal uptake of bacterial DNA or ssRNA in a chloroquine-dependent manner, MDCs are activated following cytoplasmic (non-endosomal) recognition of dsRNA mediated by the cytosolic enzyme protein kinase R (PKR) (see below).

Tab. 2: Human plasmacytoid vs. myeloid dendritic cells*

	Plasmacytoid DCs	Myeloid DCs
Relative abundance in peripheral blood	0.01-0.05 %	0.01-0.05 %
Appearance	Prominent rough endoplasmic reticulum	Numerous dendrites
Surface markers	Lin-, DR+, CD123+, CD11c-, BDCA-4+	Lin-, DR+, CD123-, C11c+
Toll-like receptors expressed	TLR7, TLR9	TLR3, TLR4, TLR8

* see references [18,19,41-43]

PDCs and MDCs express distinctive surface markers, but they differ in humans and mice. Human PDCs are lineage-, HLA-DR+, CD123+, CD11c- whereas human MDCs are lineage-, HLA-DR+, CD123-, CD11c+ cells. In the mouse, both PDCs and MDCs are CD11c+. Murine PDCs are defined as lineage-, HLA-DR+, CD11c+, Gr-1+, B220+, CD11b-. In contrast, murine MDCs are lineage-, HLA-DR+, CD11c+, Gr-1-, B220-, CD11b+ (21-23).

Innate immunity and the regulation of IFN-I production

DCs bear surface proteins critical for their activation and maturation that recognize microbial “patterns”. These receptors of the innate immune system recognize microbe-specific molecules, such as the lipopolysaccharide (LPS) of gram-negative organisms, peptidoglycans, flagellin, and microbial nucleic acids [24]. The best known are cell surface proteins homologous to the Drosophila Toll protein. The 10 known mammalian Toll-like receptors (TLRs) each consist of an extracellular domain with leucine-rich repeats, a C-terminal flanking region, a membrane-spanning domain, and a cytoplasmic Toll/IL-1 receptor homology domain or TIR, which mediates signalling following engagement of the appropriate ligand [24]. The TIR domain interacts with one or more adapter proteins, including MyD88, TIRAP/Mal [25,26], TRAM, and

TICAM-1/TRIF [27,28] (Fig. 1, Tab. 3). The adapter protein MyD88 is a key element of the signalling pathway for many of the TLRs [24,29].

Tab. 3: Human Toll-like receptors that stimulate Type I interferon production

TLR	Known ligands	Signaling pathway	References
3	dsRNA	TRIF/IRF3	[115]
4	Lipopolysaccharide	TRIF/IRF3	[116-118]
7	Imiquimod	MyD88/NFκB	[37,38]
8	Imiquimod, GU-rich ssRNA	MyD88/NFκB	[37,38]
9	Unmethylated CpG DNA	MyD88/NFκB	[44]

TLR regulation of TNFα, IL-1 and IL-6

Signal transduction through MyD88 recruits the IL-1 receptor associated kinase (IRAK-4), activating NFκB and MAP kinase. These factors lead to the activation of TNFα, IL-1, and IL-6 gene expression. MyD88 deficient mice are unresponsive to bacterial DNA containing unmethylated CpG motifs (TLR9 ligand), are resistant to LPS-induced shock (TLR4), and are grossly deficient in TNFα, IL-1, and IL-6 production, despite the fact that NFκB and MAP kinase activation is not abolished [30].

TLR regulation of IFN-I

Although most TLRs depend on MyD88 for signaling, TLR3 and TLR4 are unique in their ability to signal via both MyD88-dependent and MyD88-independent pathways [28,30-32] (Fig. 1). MyD88-independent signalling is dependent on the adapter protein TICAM-1/TRIF [27,28], which activates the transcription factor IRF-3 [33], leading to IFN-β production [28,32,34). The IFN-β enhancer sequence contains four positive regulatory domains: PDR I-IV [35]. IRF3 binds to PDR III and PDR I, NFκB to PDR II, and ATF-2/c-Jun to PDR IV. Together, IRF-3 and NFκB activate several “primary viral response genes” in addition to IFN-β, including IFN-α1, IP-10, and RANTES [4,8] (Fig. 1). IRF7, on the other hand, is required to activate the enhancers of other members of the IFN-I family [4] and to promote positive feedback regulation of IFN production [36].
In addition to TLR3 and TLR4 ligands, TLR9 ligands stimulate IFN-I production [Tab. 3]. However, TLR9-induced IFN production is not dependent on TICAM-1/TRIF. It was reported recently that certain single-stranded RNAs rich in GU or U sequences also stimulate IFN-I production through interactions with TLR7 and/or TLR8 [Tab. 3]. Imidazoquinolones, such as imiquimod, also engage these receptors, stimulating IFN-I production [37,38]. The pathway(s) involved in this response have not yet been fully elucidated.

Cross-talk between PKR and TLR3

The extracellular TLR3 molecule recognizes viral double stranded (ds) RNA and activates intracellular kinases including the dsRNA-dependent protein kinase (PKR), culminating in IFN expression [39]. PKR plays a critical role in innate immunity to viruses by recognizing intracellular (cytoplasmic) dsRNA intermediates produced during viral infection. IFN-inducible antiviral activity against the infections limited by Mx1 (influenza and VSV, see above) also is highly dependent on PKR, whereas PKR appears dispensable for protection against other viruses, such as vaccinia or picornaviruses [40]. Human PKR is a 68 kDa protein with a C-terminal kinase domain and an N-terminal dsRNA binding proteins. Its best-studied activity involves shutdown of protein synthesis through complex interactions with dsRNA and translation initiation factor-2a (eIF-2α), leading to decreased expression of viral messages (40). PKR initiates the activation of transcription factors NF-κB, ATF-2, STAT1, and IRF-1, as well as the p38 and JNK MAP kinases. It also interacts with Mal/TIRAP in TLR4 signalling (Fig. 1), but is not required for LPS-induced IFN-β production. Although PKR does not appear to bind directly to TLR3, recent studies indicate that upon binding of dsRNA to TLR3, TRAF6, TAK1, TAB2, and PKR are recruited to the receptor complex leading to NF-κB activation (Fig. 3). Thus, there is likely to be significant cross-talk between the TLR3 and PKR pathways. The precise role of PKR in these pathways remains incompletely understood and it is not known how the interaction of PKR with dsRNA activates IFN-I expression.

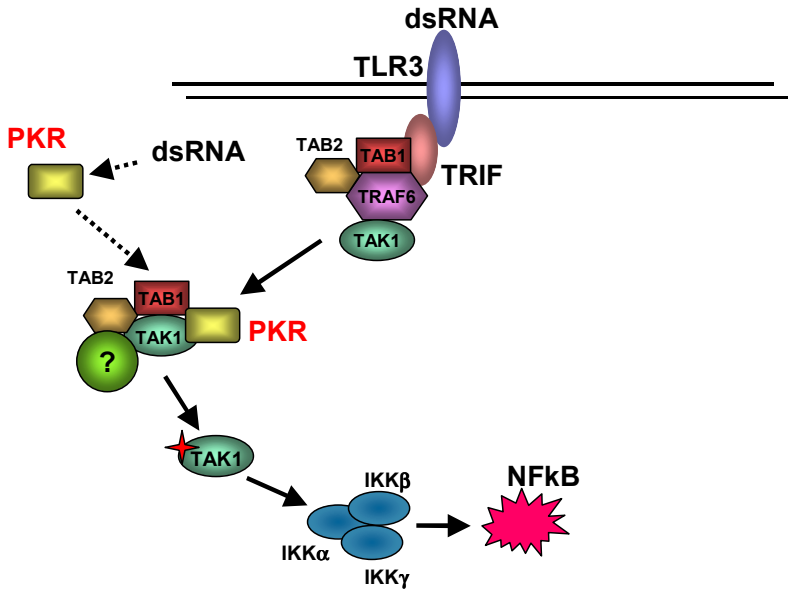


Fig. 3: Role of protein kinase R (PKR) in NFκB activation and Type I IFN production

Expression of Toll-like receptors by DC subsets

The major IFN-producing cells express different subsets of surface receptors mediating innate immunity. Specifically, PDCs express TLR7 and TLR9, whereas MDCs express TLR3, TLR4, and TLR8 [41-43]. Thus, PDCs are stimulated preferentially by viral GU-rich single-stranded RNA and unmethylated CpG DNA [37,38,44], whereas MDCs are stimulated preferentially by dsRNA and LPS [8,45]. In view of the correlation between TLR expression and responsiveness of different types of DCs to microbial stimuli, the differential expression of TLRs is likely to play a significant role in the regulation of IFN-I production by DCs [46-49]. There is some evidence that signalling from Toll-like receptors can vary depending on the cell type or maturation state. Of particular importance is the fact that macrophages express TLR3, and when stimulated with extracellular dsRNA produce IFN-I [50]. In contrast, extracellular dsRNA is incapable of stimulating IFN-I production by MDCs, which nevertheless produce high levels of IFN-I when dsRNA delivered intracellularly activates the PKR pathway [20].

Activated PDCs and MDCs home to secondary lymphoid organs

Immature DCs in the periphery sample antigen but are poor at antigen presentation due to their low expression of MHC and co-stimulatory molecules [51,52]. Immature DCs express receptors for inflammatory chemokines, allowing them to migrate to sites of inflammation where they capture antigens and undergo maturation. Immature DCs respond to the inflammatory chemokines MIP-1α, MIP-1β, MIP-3α, MIP-5, MCP-3, MCP-4, RANTES, TECK and SDF-1. Various subsets of immature DCs exhibit different chemokine responsiveness. Langerhans cells express CCR6 and migrate selectively to MIP-3α, human blood CD11c+ DC express CCR2 and migrate to MCP chemokines, and monocyte-derived DC express CCR1 and CCR5 and migrate to MIP-1α/β. Immature PDCs express CXCR4, the receptor for SDF-1, as well as lower levels of CCR2, CCR5, CCR6, and CXCR3 [53]. Thus, immature PDCs migrate to SDF-1 as well as to the IFN-γ-inducible chemokines IP-10, MIG, and ITAC [54].

DC maturation induced by TLR ligands or CD40L causes down-regulation of receptors for inflammatory chemokines and a concomitant up-regulation of receptors for chemokines expressed in the secondary lymphoid organs, especially SLC (CCL21), ELC (CCL19), and BLC (CXCL13) [55,56]. Both MDCs and PDCs up-regulate CCR7 (the receptor for SLC and ELC) upon receiving appropriate maturation signals [43]. This mediates homing to the T cell zones of secondary lymphoid tissues [55]. In contrast, the maturation of Langerhans cells leads to upregulation of CXCR5, the receptor for BLC, which may promote their selective migration to the B cell zones [56].

In the lymph nodes, IPCs are located in close proximity to the high endothelial venules (HEVs) and express of CD62L (L-selectin), which is involved in adhesion to HEVs [19]. In CD62L knockout mice, the numbers of IPCs in the paracortical areas and outer T cell zone of lymph nodes are greatly reduced. IPCs are unusual in the lymph nodes of healthy individuals, but PDCs are increased in inflammatory diseases such as tuberculosis, granulomatous lymphadenitis, Castleman's disease, and Kikuchi's disease [19,57-59].

IFN-I mediates the adjuvant effect

It has been long recognized that many protein antigens are poorly immunogenic by themselves in comparison with intact viruses or bacteria. For that reason, adjuvants are added to most subunit vaccines to take the place of immunostimulators present in the intact organism and to enhance antigen presentation by DCs. Immunological adjuvants are defined as "substances used in combination with a specific antigen that produce more immunity than the antigen alone" [60]. Effective adjuvants include TLR ligands, such as poly (I:C) (TLR3 ligand), LPS (TLR4 ligand), and CpG oligodeoxyribonucleotides (TLR9 ligand), as well as mineral salts (e.g. alum) and oil emulsions, such as incomplete Freund's adjuvant (the mineral oil Bayol F plus an emulsifier), complete Freund's adjuvant (incomplete Freund's adjuvant plus heat-killed mycobacteria), and MF59 (squalene). Importantly, the efficacy of complete Freund's adjuvant requires activity of IFN-I [61]. Moreover, IFN-I has adjuvant activity by itself, greatly augmenting antigen-specific immunoglobulin production and isotype switching when administered along with soluble antigen. IFN-I also is required for memory B cell responses. The adjuvant activity of IFN-I results from enhancement of DC maturation [61].

Experimental lupus in mice is associated with increased IFN-I production

Ten years ago, we reported that the intraperitoneal injection of pristane (2,6,10,14-tetramethylpentadecane, 0.5 ml i.p.) in BALB/c and other non-autoimmune strains of mice results in the production of autoantibodies characteristic of SLE as well as immune complex-mediated glomerulonephritis resembling lupus nephritis [62,63]. Some strains develop arthritis, as well [64]. More recently, it has become apparent that other adjuvant oils, including incomplete Freund's adjuvant and squalene (MF59 adjuvant) also can induce lupus-like disease. Following intraperitoneal injection of these adjuvant hydrocarbon oils, BALB/c and most other immunocompetent mice develop high levels of anti-Sm, anti-nRNP, anti-dsDNA, anti-ribosomal P, anti-chromatin, and anti-Su autoantibodies [65-67]. Some mice produce autoantibodies against dsRNA binding proteins such as NF90/NF45 and RNA

helicase A [68,69] as well as myositis-specific anti-OJ autoantibodies [70]. The adjuvant oil induced autoimmune syndrome is dependent on IL-6, IFN-γ, and IL-12, but not IL-4 [65,71,72] and pristane-treated mice produce large amounts of IL-6, IL-12, and TNFα in the peritoneal cavity [73]. Because the adjuvant activity of these oils is strictly dependent on intact IFN-I signaling [61], we investigated the inflammatory responses to pristane and control hydrocarbon oils that do not induce lupus. Intraperitoneal injection of pristane and other hydrocarbon oils causes a peritoneal inflammatory response followed by engulfment of the oil by phagocytic cells and the organization of polyp-like structures that have been termed “lipogranulomas” [74]. We noticed that these structures also develop in response to medicinal mineral oils, which unlike pristane do not induce lupus [73].

Pristane-induced lipogranulomas are tertiary lymphoid tissue.

We recently have found that pristane lipogranulomas are organized and function like lymphoid tissues (D. Nacionales, et al. manuscript in preparation). Immunohistochemistry revealed that the granulomas contain aggregates of B220+ B lymphocytes and CD4+ T cells as well as numerous CD11c+ DCs (Tab. 4). Moreover, some pristane lipogranulomas (but not lipogranulomas induced by medicinal mineral oil) were organized into distinct T and B cell zones. DCs were found in the T cell zones. Cells expressing chemokines characteristic of secondary lymphoid tissues (BLC, SLC, and ELC) and their respective receptors (CXCR5 and CCR7, respectively) were present in the lipogranulomas. In lymph nodes, SLC is made predominantly by high endothelial venules (HEVs) and stromal cells [75,76]. Thus, it was not surprising to find that the lipogranulomas were vascularized by blood vessels staining positively for peripheral lymph node addressin (PNAd), an HEV marker. BLC is made by follicular dendritic cells (FDCs) in the B cell follicles [77-79]. However, we have so far been unable to detect cells staining with an antibody against FDCs (FDC-M1) (D. Nacionales, unpublished data). These observations raise the possibility that BLC is produced by an alternative (non-FDC) cell type in the lipogranulomas. Studies are ongoing to identify the source of the BLC.

Germinal center-like reaction in lipogranulomas induced by pristane

Following immunization with a T cell-dependent antigen, antigen-specific B cells home to the T cell zones of secondary lymphoid tissues such as the lymph nodes and spleen where they form foci in the periarteriolar lymphoid sheath (PALS) and develop into plasmablasts. They migrate toward the lymphoid chemokines SLC (expressed by HEV) and BLC (expressed by FDC) [55]. The SLC ligand CCR7 retains B cells within the PALS, whereas expression of the BLC ligand CXCR5 permits a minority of these cells to migrate to the B cell follicles where they become organized into germinal

centers, consisting of a dark zone containing proliferating (Ki67+) centroblasts and a light zone containing non-proliferating (Ki67-) centrocytes. Centrocytes are selected for survival on the basis of their affinity for antigen and subsequently undergo further maturation into either plasma cells or memory cells [80]. Germinal centers are the sites of somatic hypermutation of the immunoglobulin variable regions, affinity maturation, and class switching [81]. FDCs capture immune complexes via Fc receptors and are thought to present antigens to the centrocytes, preventing their deletion by apoptosis. Small numbers of T cells bearing CD40 ligand found in the B cell follicles also appear to play a critical role in the survival of antigen-selected centrocytes. Individual germinal centers contain oligoclonal populations (1-3 clones) of antigen-selected B cells [82]. Germinal center B cells uniquely stain with the lectin peanut agglutinin (PNA) and with monoclonal antibody GL7 [83,84] and also express Bcl-6 [85,86]. Germinal center B cells also express antigen induced cytidine deaminase (AID), an enzyme essential for somatic hypermutation [87], and can re-express the recombination activating genes RAG-1 and RAG-2 [88]. Key aspects of the germinal center reaction are summarized in Tab. 4. Germinal centers have a lifespan of about 3 weeks, after which the antigen-specific B cells undergo apoptosis and the germinal centers atrophy [89,90].

Tab. 4: Similarities between pristane lipogranulomas and secondary lymphoid tissues

Features characteristic of secondary lymphoid tissues	Lipogranulomas
Distinct T and B cell zones	Present
Oligoclonal B cells in individual germinal centers	Present
High endothelial venules (MECA-79+)	Present
Interdigitating (CD11c+) DCs (T cell zone)	Present
Follicular DCs (FDC-M1+) (B cell zone)	Absent
Expression of BLC, SLC, and ELC	Present
B cells	
• Peanut agglutinin staining	Absent
• GL7 staining	N/A
• Ki67 staining	N/A
• Activation induced cytidine deaminase (AID) expression	Present
• Bcl-6 expression	Present
• Immunoglobulin: somatic mutation	Present
• Immunoglobulin: clonal expansion	Present

As discussed above, the organization, cellular composition, and chemokine expression pattern of pristane lipogranulomas is highly reminiscent of the germinal center reaction (Tab. 4). Individual pristane granulomas contain oligoclonal collections of B cells, some with extensive immunoglobulin variable region mutations and others with a germline configuration. In general, the latter have characteristics of B1 cells (distal V_H and D segments with a proximal J_H), whereas the mutated clones are generally more typical of conventional B cells (proximal V_H and D segments with a distal J_H) (J. Weinstein et al., unpublished data). Pristane lipogranulomas express the

germinal center B cell markers AID and Bcl-6, but so far we have been unable to detect peanut agglutinin or GL7 staining. It should be noted that FDCs have not been detected so far in pristane-induced lipogranulomas. However, it has been shown previously FDCs are not required for immunoglobulin somatic hypermutation or affinity maturation [91], two key features of the germinal center reaction. Thus, pristane-induced lipogranulomas have many similarities to individual germinal centers, but are not identical. We conclude that B cells in these structures are undergoing a germinal center-like reaction analogous to what has been seen in other instances of "lymphoid neogenesis", defined as the ectopic formation of lymphoid tissue [92]. Interestingly, ectopic lymphoid tissue in a variety of other situations is associated with humoral autoimmunity [93]. Lymphoid neogenesis is seen in the thyroid gland in Hashimoto's thyroiditis, the thymus in myasthenia gravis, the nervous system in multiple sclerosis, the salivary glands in Sjogren's syndrome, and the synovium in rheumatoid arthritis [94-97]. Ectopic lymphoid tissue may allow immunoglobulin somatic mutation to generate autoreactivity [98] in a milieu deficient in the usual censoring mechanisms that remove the self-reactive B cells arising in germinal centers.

IFN-I is produced at high levels in pristane lipogranulomas

Following immunization with exogenous antigens in adjuvant, IFN-I is produced in the draining lymph nodes [19]. This also is the case in the ectopic lymphoid tissue arising in response to pristane (D.C. Nacionales et al., submitted). IFN-inducible gene expression (e.g. Mx1, IRF-7, IP-10) is significantly higher in pristane vs. medicinal mineral oil induced lipogranulomas. The same pattern is seen in peritoneal cells from pristane vs. mineral oil treated mice. Additionally, peritoneal cells from pristane-treated mice expressed more Mx1 after stimulation with LPS or poly (I:C) than peritoneal cells from mineral oil-treated mice. Thus, pristane causes hyperresponsiveness to stimulation with TLR3 and TLR4 ligands that may contribute to the increased constitutive expression of IFN-I.

IFN-I in human autoimmune disease

There has been recent interest in the possibility that IFN-I might play a role in the pathogenesis of autoimmune diseases such as lupus. IFN-α treatment in hepatitis C infection, malignant carcinoid syndrome, and chronic myelogenous leukemia is sometimes associated with autoimmune phenomena, including sarcoidosis [99], autoimmune thyroiditis, and autoimmune hepatitis [100]. The induction of antinuclear antibodies and anti-dsDNA antibodies has been reported and there are case reports of SLE [101-103]. The serum level of IFN-α has been reported to correlate with anti-dsDNA antibody levels and disease activity in SLE [104-106] and increased IFN-I expression is seen in lupus skin lesions [107]. Moreover, as discussed above, recent studies point

to an IFN-I gene expression “signature” associated with SLE [9,10]. In view of these observations and our own observations in experimental murine lupus, we examined the regulation of IFN-I in SLE patients.

Regulation of IFN-I production in SLE patients

As in the peritoneal exudate and lipogranulomas from pristane-treated mice, we used the expression of Mx1 and other IFN-inducible genes to demonstrate increased expression of IFN-I by PBMCs from SLE patients vs. patients with other systemic autoimmune diseases or normal controls (H. Zhuang et al., submitted). There was an association between increased IFN-I levels and the production of autoantibodies against the U small ribonucleoproteins (snRNPs) (anti-Sm and anti-RNP antibodies), the Y1-4 ribonucleoproteins (anti-Ro60 antibodies), and double-stranded DNA. Strikingly, there was a strong inverse association between the production of anti-phospholipid autoantibodies and high levels of IFN-I, suggesting that the latter are regulated differently than autoantibodies to ribonucleoprotein and deoxyribonucleoprotein autoantigens. Interestingly, by flow cytometry the numbers of both PDCs and MDCs were reduced in SLE patients in comparison with healthy controls, despite the high systemic levels of IFN-I (H. Zhuang et al., submitted). In contrast to peritoneal cells from pristane-treated mice, PBMCs from SLE patients are hypo-responsive to TLR3 and TLR4 ligands. We hypothesize that the cells producing IFN-I, which in SLE may include TLR3+/TLR4+ MDCs, may migrate to sites of inflammation where they continue to produce IFN-I, activating the expression of Mx1 and other IFN-inducible genes in circulating PBMCs. This may leave the peripheral blood relatively deficient in IPCs. Notably, there is a very similar picture (low PDC and MDC numbers) in HIV infected patients [108]. Conversely, inflammatory sites (e.g. the peritoneal cavity of pristane-treated mice) and/or secondary lymphoid organs may be enriched in these cells. We speculate that this (and the inability of mature PDCs and MDCs to re-circulate once they have homed to the secondary lymphoid organs may contribute to the increased susceptibility of lupus patients to bacterial and viral infections [109,110] as well as to their poor humoral responses to vaccines [111,112]. Indeed, the production of polyclonal IgG antibodies to influenza virus is abrogated when PDCs are depleted [113] and IFN-I is known to influence the balance of immunoglobulin isotypes produced in response to polyclonal [114] or antigen-specific [61] immune stimulation.

Conclusion

There is considerable interest in the potential role of IFN-I in SLE. Exogenous administration of IFN-α can induce autoimmune diseases, including lupus, suggesting that there may be a cause and effect relationship. We have found increased expression of IFN-I inducible genes in both SLE patients and in an experimental model of lupus in mice. In both cases, there was dysregulation

of responses to TLR3 and TLR4 ligands, consistent with abnormalities in either the function or distribution of MDCs. Flow cytometry revealed a depletion of MDCs as well as PDCs in the peripheral blood of SLE patients. The peritoneal cavity of pristane-treated mice, in contrast, contained large numbers of MDCs. Finally, there was a strong association between increased IFN-I production and the production of certain autoantibodies characteristic of SLE, including anti-nRNP/Sm, anti-Ro60, and anti-dsDNA. We are investigating the possibility that the association of cellular TLR3 or TLR9 ligands with these self-antigens may promote the maturation of DCs presenting them by stimulating IFN-I production.

References

1. Sen GC, Lengyel P. The interferon system. J Biol Chem 1992; 267(8):5017-5020.
2. Diaz MO, Pomykala HM, Bohlander SK, Maltepe E, Malik K, Brownstein B et al. Structure of the human type-I interferon gene cluster determined from a YAC clone contig. Genomics 1994; 22(3):540-552.
3. Barnes B, Lubyova B, Pitha PM. On the role of IRF in host defense. J Interferon Cytokine Res 2002; 22(1):59-71.
4. Lin R, Genin P, Mamane Y, Hiscott J. Selective DNA binding and association with the CREB binding protein coactivator contribute to differential activation of alpha/beta interferon genes by interferon regulatory factors 3 and 7. Mol Cell Biol 2000; 20(17):6342-6353.
5. Taniguchi T, Takaoka A. A weak signal for strong responses: interferon-alpha/beta revisited. Nat Rev Mol Cell Biol 2001; 2(5):378-386.
6. Kotenko SV, Pestka S. Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. Oncogene 2000; 19(21):2557-2565.
7. Sen GC, Ransohoff RM. Interferon-induced antiviral actions and their regulation. Adv Virus Res 1993; 42:57-102.
8. Doyle S, Vaidya S, O'Connell R, Dadgostar H, Dempsey P, Wu T et al. IRF3 mediates a TLR3/TLR4-specific antiviral gene program. Immunity 2002; 17(3):251-263.
9. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 2003; 197(6):711-723.
10. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 2003; 100(5):2610-2615.
11. MacQuillan GC, Mamotte C, Reed WD, Jeffrey GP, Allan JE. Upregulation of endogenous intrahepatic interferon stimulated genes during chronic hepatitis C virus infection. J Med Virol 2003; 70(2):219-227.
12. Staeheli P, Pitossi F, Pavlovic J. Mx proteins: GTPases with antiviral activity. Trends Cell Biol 1993; 3(8):268-272.
13. Zhang L, Pagano JS. Structure and function of IRF-7. J Interferon Cytokine Res 2002; 22(1):95-101.
14. Benech P, Merlin G, Revel M, Chebath J. 3' end structure of the human (2'-5') oligo A synthetase gene: prediction of two distinct proteins with cell type-specific expression. Nucleic Acids Res 1985; 13(4):1267-1281.

15. Rebouillat D, Hovanessian AG. The human 2',5'-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties. J Interferon Cytokine Res 1999; 19(4):295-308.
16. Castelli J, Wood KA, Youle RJ. The 2-5A system in viral infection and apoptosis. Biomed Pharmacother 1998; 52(9):386-390.
17. Ronnblom L, Ramstedt U, Alm GV. Properties of human natural interferon-producing cells stimulated by tumor cell lines. Eur J Immunol 1983; 13(6):471-476.
18. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S et al. The nature of the principal type 1 interferon-producing cells in human blood. Science (Wash D C) 1999; 284(5421):1835-1837.
19. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat Med 1999; 5(8):919-923.
20. Diebold SS, Montoya M, Unger H, Alexopoulou L, Roy P, Haswell LE et al. Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. Nature (Lond) 2003; 424(6946):324-328.
21. Asselin-Paturel C, Boonstra A, Dalod M, Durand I, Yessaad N, Dezutter-Dambuyant C et al. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat Immunol 2001; 2(12):1144-1150.
22. Nakano H, Yanagita M, Gunn MD. Cd11c(+)b220(+)gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. J Exp Med 2001; 194(8):1171-1178.
23. Bjorck P. Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor-treated mice. Blood 2001; 98(13):3520-3526.
24. Kaisho T, Akira S. Toll-like receptors as adjuvant receptors. Biochim Biophys Acta 2002; 1589(1):1-13.
25. Horng T, Barton GM, Medzhitov R. TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol 2001; 2(9):835-841.
26. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G et al. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature (Lond) 2001; 413(6851):78-83.
27. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science (Wash D C) 2003; 301(5633):640-643.
28. Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T. TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. Nat Immunol 2003; 4(2):161-167.
29. Akira S, Yamamoto M, Takeda K. Role of adapters in Toll-like receptor signalling. Biochem Soc Trans 2003; 31(Pt 3):637-642.
30. Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 1999; 11(1):115-122.
31. Kaisho T, Takeuchi O, Kawai T, Hoshino K, Akira S. Endotoxin-induced maturation of MyD88-deficient dendritic cells. J Immunol 2001; 166(9):5688-5694.
32. Toshchakov V, Jones BW, Perera PY, Thomas K, Cody MJ, Zhang S et al. TLR4, but not TLR2, mediates IFN-beta-induced STAT1alpha/beta-dependent gene expression in macrophages. Nat Immunol 2002; 3(4):392-398.
33. Kawai T, Takeuchi O, Fujita T, Inoue J, Muhlradt PF, Sato S et al. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol 2001; 167(10):5887-5894.

34. Matsumoto M, Kikkawa S, Kohase M, Miyake K, Seya T. Establishment of a monoclonal antibody against human Toll-like receptor 3 that blocks double-stranded RNA-mediated signaling. Biochem Biophys Res Commun 2002; 293(5):1364-1369.
35. Maniatis T, Falvo JV, Kim TH, Kim TK, Lin CH, Parekh BS et al. Structure and function of the interferon-beta enhanceosome. Cold Spring Harb Symp Quant Biol 1998; 63:609-620.
36. Dalod M, Salazar-Mather TP, Malmgaard L, Lewis C, Asselin-Paturel C, Briere F et al. Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. J Exp Med 2002; 195(4):517-528.
37. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science (Wash D C) 2004; 303(5663):1526-1529.
38. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science (Wash D C) 2004; 303(5663):1529-1531.
39. Jiang Z, Zamanian-Daryoush M, Nie H, Silva AM, Williams BR, Li X. Poly(dI x dC)-induced Toll-like receptor 3 (TLR3)-mediated activation of NFkappa B and MAP kinase is through an interleukin-1 receptor-associated kinase (IRAK)-independent pathway employing the signaling components TLR3-TRAF6-TAK1-TAB2-PKR. J Biol Chem 2003; 278(19):16713-16719.
40. Brierley MM, Fish EN. Review: IFN-alpha/beta receptor interactions to biologic outcomes: understanding the circuitry. J Interferon Cytokine Res 2002; 22(8):835-845.
41. Kadowaki N, Ho S, Antonenko S, de Waal MR, Kastelein RA, Bazan F et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. J Exp Med 2001; 194(6):863-870.
42. Krug A, Towarowski A, Britsch S, Rothenfusser S, Hornung V, Bals R et al. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur J Immunol 2001; 31(10):3026-3037.
43. Jarrossay D, Napolitani G, Colonna M, Sallusto F, Lanzavecchia A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. Eur J Immunol 2001; 31(11):3388-3393.
44. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H et al. A Toll-like receptor recognizes bacterial DNA. Nature (Lond) 2000; 408(6813):740-745.
45. Doyle SE, O'Connell R, Vaidya SA, Chow EK, Yee K, Cheng G. Toll-like receptor 3 mediates a more potent antiviral response than Toll-like receptor 4. J Immunol 2003; 170(7):3565-3571.
46. Bauer M, Redecke V, Ellwart JW, Scherer B, Kremer JP, Wagner H et al. Bacterial CpG-DNA triggers activation and maturation of human CD11c-, CD123+ dendritic cells. J Immunol 2001; 166(8):5000-5007.
47. Cella M, Salio M, Sakakibara Y, Langen H, Julkunen I, Lanzavecchia A. Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. J Exp Med 1999; 189:821-829.
48. Hartmann G, Weiner GJ, Krieg AM. CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells. Proc Natl Acad Sci U S A 1999; 96(16):9305-9310.
49. Kadowaki N, Antonenko S, Liu YJ. Distinct CpG DNA and polyinosinic-polycytidylic acid double-stranded RNA, respectively, stimulate CD11c- type 2

dendritic cell precursors and CD11c+ dendritic cells to produce type I IFN. J Immunol 2001; 166(4):2291-2295.
50. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature (Lond) 2001; 413(6857):732-738.
51. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature (Lond) 1998; 392:245-252.
52. Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N. Antigen-specific Inhibition of Effector T Cell Function in Humans after Injection of Immature Dendritic Cells. J Exp Med 2001; 193(2):233-238.
53. Zou W, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Med 2001; 7(12):1339-1346.
54. Caux C, Ait-Yahia S, Chemin K, de Bouteiller O, Dieu-Nosjean MC, Homey B et al. Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. Springer Semin Immunopathol 2000; 22(4):345-369.
55. Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 1999; 99(1):23-33.
56. Saeki H, Wu MT, Olasz E, Hwang ST. A migratory population of skin-derived dendritic cells expresses CXCR5, responds to B lymphocyte chemoattractant in vitro, and co-localizes to B cell zones in lymph nodes in vivo. Eur J Immunol 2000; 30(10):2808-2814.
57. Facchetti F, Wolf-Peeters C, van den Oord JJ, de Vos R, Desmet VJ. Plasmacytoid monocytes (so-called plasmacytoid T-cells) in Kikuchi's lymphadenitis. An immunohistologic study. Am J Clin Pathol 1989; 92(1):42-50.
58. Facchetti F, Wolf-Peeters C, de Vos R, van den Oord JJ, Pulford KA, Desmet VJ. Plasmacytoid monocytes (so-called plasmacytoid T cells) in granulomatous lymphadenitis. Hum Pathol 1989; 20(6):588-593.
59. Facchetti F, Wolf-Peeters C, van den Oord JJ, Desmet VJ. Plasmacytoid monocytes (so-called plasmacytoid T cells) in Hodgkin's disease. J Pathol 1989; 158(1):57-65.
60. Ramon G. Sur la toxine et sur l'anatoxine diphtheriques. Ann Inst Pasteur 1924; 38:1-10.
61. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 2001; 14(4):461-470.
62. Satoh M, Reeves WH. Induction of lupus-associated autoantibodies in BALB/c mice by intraperitoneal injection of pristane. J Exp Med 1994; 180:2341-2346.
63. Satoh M, Kumar A, Kanwar YS, Reeves WH. Antinuclear antibody production and immune complex glomerulonephritis in BALB/c mice treated with pristane. Proc Natl Acad Sci USA 1995; 92:10934-10938.
64. Wooley PH, Seibold JR, Whalen JD, Chapdelaine JM. Pristane-induced arthritis. The immunologic and genetic features of an experimental murine model of autoimmune disease. Arthritis Rheum 1989; 32:1022-1030.
65. Richards HB, Satoh M, Shaw M, Libert C, Poli V, Reeves WH. IL-6 dependence of anti-DNA antibody production: evidence for two pathways of autoantibody formation in pristane-induced lupus. J Exp Med 1998; 188:985-990.
66. Satoh M, Richards HB, Shaheen VM, Yoshida H, Shaw M, Naim JO et al. Widespread susceptibility among inbred mouse strains to the induction of lupus autoantibodies by pristane. Clin Exp Immunol 2000; 121:399-405.

67. Satoh M, Hamilton KJ, Ajmani AK, Dong X, Wang J, Kanwar YS et al. Autoantibodies to ribosomal P antigens with immune complex glomerulonephritis in SJL mice treated with pristane. J Immunol 1996; 157:3200-3206.
68. Satoh M, Shaheen VM, Kao PN, Okano T, Shaw M, Yoshida H et al. Autoantibodies define a family of proteins with conserved double-stranded RNA binding domains as well as DNA-binding activity. J Biol Chem 1999; 274:34598-34604.
69. Yoshida H, Satoh M, Behney KM, Lee CG, Richards HB, Shaheen VM et al. Effect of an exogenous trigger on the pathogenesis of lupus in NZB X NZW (F1) mice. Arthritis Rheum 2002; 46:2235-2244.
70. Yang JQ, Singh AK, Wilson MT, Satoh M, Stanic AK, Park JJ et al. Immunoregulatory role of CD1d in the hydrocarbon oil-induced model of lupus nephritis. J Immunol 2003; 171(4):2142-2153.
71. Richards HB, Satoh M, Jennette JC, Croker BP, Reeves WH. Interferon-gamma promotes lupus nephritis in mice treated with the hydrocarbon oil pristane. Kidney Int 2001; 60:2173-2180.
72. Calvani N, Satoh M, Croker BP, Reeves WH, Richards HB. Nephritogenic autoantibodies but absence of nephritis in Il-12p35-deficient mice with pristane-induced lupus. Kidney Int 2003; 64:897-905.
73. Satoh M, Kuroda Y, Yoshida H, Behney KM, Mizutani A, Akaogi J et al. Induction of lupus autoantibodies by adjuvants. J Autoimm 2003; 21:1-9.
74. Shaheen VM, Satoh M, Richards HB, Yoshida H, Shaw M, Jennette JC et al. Immunopathogenesis of environmentally induced lupus in mice. Environ Health Perspect 1999; 107(Suppl 5):723-727.
75. Baekkevold ES, Yamanaka T, Palframan RT, Carlsen HS, Reinholt FP, von Andrian UH et al. The CCR7 ligand elc (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. J Exp Med 2001; 193(9):1105-1112.
76. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. Proc Natl Acad Sci U S A 1998; 95(1):258-263.
77. Gunn MD, Ngo VN, Ansel KM, Ekland EH, Cyster JG, Williams LT. A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. Nature (Lond) 1998; 391:799-803.
78. Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature (Lond) 2000; 406(6793):309-314.
79. Cyster JG, Ansel KM, Reif K, Ekland EH, Hyman PL, Tang HL et al. Follicular stromal cells and lymphocyte homing to follicles. Immunol Rev 2000; 176:181-193.
80. Choe J, KIm HS, Armitage RJ, Choi YS. The functional role of B cell antigen receptor stimulation and IL-4 in the generation of human memory B cells from germinal center B cells. J Immunol 1997; 159(8):3757-3766.
81. Liu YJ, Malisan F, de Bouteiller O, Guret C, Lebecque S, Banchereau J et al. Within germinal centers, isotype switching of immunoglobulin genes occurs after the onset of somatic mutation. Immunity 1996; 4(3):241-250.
82. Jacob J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3- nitrophenyl)acetyl. II. A common clonal origin for periarteriolar lymphoid sheath-associated foci and germinal centers. J Exp Med 1992; 176(3):679-687.
83. Han S, Zheng B, Takahashi Y, Kelsoe G. Distinctive characteristics of germinal center B cells. Semin Immunol 1997; 9:255-260.

84. Kelsoe G. Life and death in germinal centers (redux). Immunity 1996; 4(2):107-111.
85. Dent AL, Shaffer AL, Yu X, Allman D, Staudt LM. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. Science (Wash D C) 1997; 276(5312):589-592.
86. Ye BH, Cattoretti G, Shen Q, Zhang J, Hawe N, de Waard R et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nature Genetics 1997; 16:161-170.
87. Muramatsu M, Sankaranand VS, Anant S, Sugai M, Kinoshita K, Davidson NO et al. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J Biol Chem 1999; 274(26):18470-18476.
88. Giachino C, Padovan E, Lanzavecchia A. Re-expression of RAG-1 and RAG-2 genes and evidence for secondary rearrangements in human germinal center B lymphocytes. Eur J Immunol 1998; 28(11):3506-3513.
89. Kosco-Vilbois MH, Zentgraf H, Gerdes J, Bonnefoy JY. To 'B' or not to 'B' a germinal center? Immunol Today 1997; 18(5):225-230.
90. Han S, Zheng B, Dal Porto J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl IV. Affinity-dependent, antigen-driven B cell apoptosis in germinal centers as a mechanism for maintaining self-tolerance. J Exp Med 1995; 182:1635-1644.
91. Hannum LG, Haberman AM, Anderson SM, Shlomchik MJ. Germinal center initiation, variable gene region hypermutation, and mutant B cell selection without detectable immune complexes on follicular dendritic cells. J Exp Med 2000; 192(7):931-942.
92. Kratz A, Campos-Neto A, Hanson MS, Ruddle NH. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. J Exp Med 1996; 183:1461-1472.
93. Hjelmstrom P. Lymphoid neogenesis: de novo formation of lymphoid tissue in chronic inflammation through expression of homing chemokines. J Leukoc Biol 2001; 69(3):331-339.
94. Stott DI, Hiepe F, Hummel M, Steinhauser G, Berek C. Antigen-driven clonal proliferation of B cells within the target tissue of an autoimmune disease. The salivary glands of patients with Sjogren's syndrome. J Clin Invest 1998; 102(5):938-946.
95. Xanthou G, Polihronis M, Tzioufas AG, Paikos S, Sideras P, Moutsopoulos HM. "Lymphoid" chemokine messenger RNA expression by epithelial cells in the chronic inflammatory lesion of the salivary glands of Sjogren's syndrome patients: possible participation in lymphoid structure formation. Arthritis Rheum 2001; 44(2):408-418.
96. Schroder AE, Greiner A, Seyfert C, Berek C. Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. Proc Natl Acad Sci U S A 1996; 93(1):221-225.
97. Randen I, Mellbye OJ, Forre O, Natvig JB. The identification of germinal centres and follicular dendritic cell networks in rheumatoid synovial tissue. Scand J Immunol 1995; 41(5):481-486.
98. Diamond B, Scharff MD. Somatic mutation of the T15 heavy chain gives rise to antibody with autoantibody specificity. Proc Natl Acad Sci USA 1984; 81:5841-5844.
99. Fiorani C, Sacchi S, Bonacorsi G, Cosenza M. Systemic sarcoidosis associated with interferon-alpha treatment for chronic myelogenous leukemia. Haematologica 2000; 85(9):1006-1007.

100. Dumoulin FL, Leifeld L, Sauerbruch T, Spengler U. Autoimmunity induced by interferon-alpha therapy for chronic viral hepatitis. Biomed Pharmacother 1999; 53(5-6):242-254.
101. Ioannou Y, Isenberg DA. Current evidence for the induction of autoimmune rheumatic manifestations by cytokine therapy. Arthritis Rheum 2000; 43:1431-1442.
102. Kalkner KM, Ronnblom L, Karlsson Parra AK, Bengtsson M, Olsson Y, Oberg K. Antibodies against double-stranded DNA and development of polymyositis during treatment with interferon. Q J Med 1998; 91(6):393-399.
103. Ronnblom LE, Alm GV, Oberg KE. Autoimmunity after alpha-interferon therapy for malignant carcinoid tumors [see comments]. Ann Intern Med 1991; 115(3):178-183.
104. Kim T, Kanayama Y, Negoro N, Okamura M, Takeda T, Inoue T. Serum levels of interferons in patients with systemic lupus erythematosus. Clin Exp Immunol 1987; 70(3):562-569.
105. Hooks JJ, Moutsopoulos HM, Geis SA, Stahl NI, Decker JL, Notkins AL. Immune interferon in the circulation of patients with autoimmune disease. N Engl J Med 1979; 301(1):5-8.
106. Preble OT, Black RJ, Friedman RM, Klippel JH, Vilcek J. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. Science (Wash D C) 1982; 216(4544):429-431.
107. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 2001; 159(1):237-243.
108. Pacanowski J, Kahi S, Baillet M, Lebon P, Deveau C, Goujard C et al. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. Blood 2001; 98(10):3016-3021.
109. Abramson SB, Kramer SB, Radin A, Holzman R. Salmonella bacteremia in systemic lupus erythematosus. Arthritis Rheum 1985; 28:75-79.
110. Reveille JD, Bartolucci A, Alarcon GS. Prognosis in systemic lupus erythematosus. Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. Arthritis Rheum 1990; 33(1):37-48.
111. Gottlieb AB, Lahita RG, Chiorazzi N, Kunkel HG. Immune function in systemic lupus erythematosus: Impairment of in vitro T-cell proliferation and in vivo antibody response to exogenous antigen. J Clin Invest 1979; 63:885-892.
112. Jarrett M, Schiffman G, Barland P, Grayzel A. Impaired response to pneumococcal vaccine in systemic lupus erythematosus. Arthritis Rheum 1980; 23:1287-1293.
113. Jego G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity 2003; 19(2):225-234.
114. Finkelman FD, Svetic A, Gresser I, Snapper C, Holmes J, Trotta PP et al. Regulation by interferon alpha of immunoglobulin isotype selection and lymphokine production in mice. J Exp Med 1991; 174(5):1179-1188.
115. Fagarasan S, Shinkura R, Kamata T, Nogaki F, Ikuta K, Tashiro K et al. Alymphoplasia (aly)-type nuclear factor kappaB-inducing kinase (NIK) causes defects in secondary lymphoid tissue chemokine receptor signaling and homing of peritoneal cells to the gut-associated lymphatic tissue system. J Exp Med 2000; 191(9):1477-1486.
116. Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P et al. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4) [see

comments] [published erratum appears in J Exp Med 1999 May 3;189(9):following 1518]. J Exp Med 1999; 189(4):615-625.
117. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y et al. Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J Immunol 1999; 162:3749-3752.
118. Poltorak A, He X, Smirnova I, Liu MY, van Huffel C, Du X et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science (Wash D C) 1998; 282(5396):2085-2088.

Abbreviations used in this manuscript

IFN, interferon; IFN-I, Type I interferon; IPC, interferon producing cell; PDC, plasmacytoid dendritic cell; MDC, myeloid dendritic cell; DC, dendritic cell; HEV, high endothelial venule, IFNAR, interferon a receptor (Type interferon receptor); IRF, interferon regulatory factor; OAS, oligoadenylate synthase; FDC, follicular dendritic cell

Research Support

This work was supported by research grants R01-AR40391, R01-AR44731, K08-DK02890 (HBR), and M01 R00082 from the US Public Health Service, by funding from the State of Florida to the University of Florida Center for Autoimmune Diseases, and by generous gifts from Lupus Link, Inc. (Daytona Beach, FL) and Mr. Lewis M. Schott.

Acknowledgments

We thank Drs. Nicholas Chiorazzi and Xiao-Jie Yan for helpful discussions.