A Pivotal Role for the Natural Interferon α–producing Cells (Plasmacytoid Dendritic Cells) in the Pathogenesis of Lupus

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The human type I IFN gene family consists of 13 genes encoding IFN-α subtypes and one gene each for IFN-β and -ω. The type I IFN proteins show similarities with respect to both structure and function; for instance, they are typically produced by cells exposed to virus and interact with the same receptor, the IFNAR (I). The type I IFNs have well established direct antiviral and antiproliferative effects, but also several prominent immunoregulatory actions that have come into focus in recent years (1–3). The latter include the ability to promote survival and differentiation of antigen-activated Th1 cells. These effects of type I IFN may, in part, be due to their ability to activate signal transducer and activator of transcription (STAT) 4 and maintain expression of a functional IL-12R. Type I IFN can also cause maturation of efficiently antigen-presenting monocyte-derived dendritic cells (DCs) and stimulate B lymphocytes (4, 5).

The IFN-α–producing Cells. While many cell types produce type I IFN in vitro when exposed to double-stranded (ds)RNA and some RNA viruses, a specialized leukocyte is responsible for the IFN-α production induced by a wider spectrum of agents, including viruses, bacteria, protozoa, certain cell lines, and also unmethylated CpG-DNA (6–8). This major IFN-α–producing cell (IPC) was early on designated natural IPC (NIPC) and subsequent work (for a review see reference 6) revealed that NIPCs were infrequent (≈0.1% of PBMCs) but very productive on a per cell basis (≈10 pg IFN-α per cell). The expression of the IFN-α/β genes encoded in NIPCs was markedly dependent on costimulation (“priming”) of the cells by cytokines, in particular IL-3, GM-CSF, and type I IFNs. These cells lacked lineage specific surface antigens, but expressed MHC class II (for a review, see reference 6). The NIPCs were shown to express, e.g., CD4, CD36, CD40, CD44, CD45RA, and CD83, but lacked CD80, CD86, and CD11c, suggesting they were immature DCs (9). Their phenotype was in fact similar to a subsequently identified DC precursor (10, 11), now often referred to as plasmacytoid DCs (PDCs). Such purified PDCs were also shown to contain the NIPC population (12, 13). While mature PDCs were originally shown to induce Th2 cell development, they can efficiently promote Th1 immune responses in vitro to antigens associated with type I IFN induction (14, 15). The latter may occur especially in viral infections, but because the NIPCs/PDCs express toll-like receptor 9 (TLR9) that recognizes bacterial unmethylated CpG-DNA (16, 17), they could also be important in bacterial infections. In fact, simultaneous ligation of CD40 on NIPCs/PDCs and activation by CpG-DNA induce the production of high levels of both IFN-α and IL-12 (16), both cytokines important in Th1 immune responses.

The lack of specific markers directly identifying NIPCs/PDCs, has hampered the studies of these cells. A great step forward was the demonstration of two novel markers, BDCA-2 and BDCA-4, present on ≈0.4% of PBMCs (18). These antigens seem to be unique for PDCs in peripheral blood, although BDCA-2 is lost as the cells mature and BDCA-4 also appears on differentiating monocyte-derived and CD34&sup+&nbsp; cell-derived DCs (18). In this way, for the first time, the PDCs can be positively identified. One important question is the identity and function of the BDCA-2 and -4 molecules. In this issue, Dzionek and coworkers report on the molecular cloning and characterization of BDCA-2 and show that this molecule represents a unique endocytic type II single-CRD C-type lectin, able to target ligands into the antigen processing and peptide-loading compartment (19). Whether this is the physiological function of BDCA-2 remains to be established, and it seems important to identify the natural ligand(s) to this molecule. Perhaps the most interesting finding in the paper by Dzionek et al. is the observation that ligation of BDCA-2 inhibit the IFN-α production by NIPCs/PDCs triggered by a wide variety of different IFN-α inducers. It is intriguing that such PDCs still can mature into efficient antigen presenters despite downregulated IFN-α production (19). Therefore, as suggested by Dzionek and coworkers, the BDCA-2 molecule and any endogenous or microbial ligands may have an important role in immune activation by favoring...
Th2 immune responses. Another question is therefore if BDCA-2 ligation can cause inhibition of other functions of NIPCs/PDCs, such as IL-12 production.

**Type I IFN and Autoimmunity.** One important issue is the biological relevance of the NIPCs/PDCs and their produced cytokines in man. This is true both with regard to the normal immune response to infectious microorganisms and in pathological immune responses such as autoimmunity. These questions are difficult to approach experimentally in mice, because type I IFNs do not activate the murine Th1 pathway due to a deficiency in the activation of STAT4 (20). Some lessons regarding the relation between IFN-α and autoimmunity can, however, be learned from the clinic, where IFN-α has been used as a therapeutic agent for more than two decades. A prominent side effect in IFN-α–treated individuals is the development of autoantibodies, and as many as 19% of the patients have been reported to develop an autoimmune disease (21, 22). Both organ-specific and nonorgan-specific autoimmune diseases have been observed. Among the latter, development of systemic lupus erythematosus (SLE) during IFN-α therapy was reported already in 1990 (23). Subsequently, it was shown that in the course of IFN-α therapy of patients with carcinoid tumors, as many as 22% develop antinuclear autoantibodies (ANAs), 8% anti-dsDNA antibodies, and 0.7% SLE (21, 24). These observations obviously raise the question if not the type I IFN system could be involved in the etiopathogenesis of naturally occurring SLE, the prototype systemic autoimmune disease that is characterized by the presence of a wide variety of autoantibodies and inflammation in many organs and tissues.

**Evidence for a Role of the Type I IFN System in SLE.** Early studies of the immune system in SLE patients revealed increased serum levels of IFN, subsequently characterized as IFN-α, that were suggested to be of importance in the disease process (25). The serum IFN-α levels in SLE are actually sometimes as high as in patients with acute viral infections or after therapeutic injections of large IFN-α doses. Also, several symptoms in SLE mimic those in influenza or during IFN-α therapy, for instance fever, myalgia, and arthralgia. Such observations were however largely neglected by lupus immunologists. In recent years several novel findings have been made regarding the possible mechanisms behind the ongoing IFN-α production in SLE and its cellular basis, that have renewed the interest in the area and opened a new field in lupus research. One first observation was that blood of SLE patients frequently contained an inducer of IFN-α production by normal PBMCs, consisting of anti-dsDNA antibodies, and DNA in complex (26, 27). Interestingly, this IFN-α–inducing factor in SLE (SLE-IIF) selectively activated the IFN-α synthesis in NIPCs/PDCs (27) and the same cells were also activated in vitro by autoantibodies combined with unmethylated CpG-containing plasmid DNA (7) or material released by apoptotic cells (28, 29). With regard to SLE-IIF and the combination of autoantibodies and apoptotic cells, the FcγRII (CD 32) is necessary for induction of IFN-α production, because the latter was blocked by anti-FcγRII antibodies and Fc-fragments or aggregated Ig, while the F(ab)’2 portion of autoantibodies lost stimulatory activity (30; unpublished data). Furthermore, antibodies against CD16 and CD64 were without effect. This suggests that NIPCs/PDCs do express FcγRII, although their presence on the cells has not been directly demonstrated. An in vivo role for NIPCs/PDCs in the IFN-α production in SLE is supported by the finding of sharply reduced numbers of these cells in blood (26) and presence of activated NIPCs/PDCs in the skin of SLE patients (31, 32). The noted reduction of circulating NIPCs/PDCs in SLE patients was recently confirmed and it was also observed that monocytes in SLE blood can act as antigen-presenting cells (33). Monocyte differentiation into DCs in vitro was achieved by SLE serum and was due to its content of IFN-α, underscoring the importance of this cytokine in early immune activation (4) and consequently also the autoimmune process in SLE. Finally, the activation of the type I IFN system in SLE correlates with both disease activity and severity (34).

**Mechanism for the Activation and Action of the Type I IFN System in SLE.** We have formulated a hypothesis that explains the activation and pivotal action of the type I IFN system in the development of SLE (Fig. 1; see also reference 35). It is based on the initial formation of autoantibodies against nucleic acid and associated proteins derived from apoptotic cells that occur during infections because of costimulatory effects of type I IFN and other cytokines. This may occur regularly, but is usually self-limiting and does not cause autoimmune disease. However, in certain genetically predisposed individuals, immune complexes consisting of autoantibodies and cell-derived nucleic acid–containing material continue to activate IFN-α production as endogenous IFN-α inducers, even when the infectious microorganisms have been eliminated. The IFN-α production occurs in the NIPCs/PDCs and the prolonged IFN-α production in these cells will sustain the autoimmune process, resulting in generation of more autoantibodies and IFN-α inducers. This is facilitated by the fact that antigen presentation and IFN-α production take place in similar if not identical DCs and that the IFN-α inducers and autoantigen are in the same molecular complex. Consequently, a vicious circle is established, the activity of which can be augmented by the recruitment of new NIPCs/PDCs to tissues, by priming of these cells by IFN-α and other cytokines, by generation of more endogenous IFN-α inducers from apoptotic cells, and by formation of more autoantibodies. Several proposed pathogenetic factors in lupus may also support such a vicious circle, such as decreased nuclease activity, increased apoptosis in affected tissues and decreased scavenging of apoptotic material and immune complexes.

**Therapeutic Targets in the Type I IFN System.** The proposed etiopathogenic role of the type I IFN system in SLE (Fig. 1) indicates several ways by which the IFN-α production could be inhibited by new therapeutic agents. First, the endogenous IFN-α inducers can be targeted. They can for instance be degraded by nucleases or, alternatively, their
interaction with NIPCs/PDCs via, e.g., FcγRII could be inhibited. Second, the action of produced IFN-α can be prevented by the use of neutralizing anti–IFN-α antibodies, soluble IFNAR, or antibodies blocking the anti–IFNAR. Third, the actual IFN-α-producing cells, the NIPCs/PDCs, would be an attractive target and the IFN-α gene expression in the cells could be inhibited. The observation by Dzionek and coworkers (19) that ligation of BDCA-2 with antibodies abolishes the ability of NIPCs/PDCs to produce IFN-α is in this context indeed exciting. Such anti–BDCA-2 antibodies inhibit the response to both a viral inducer and inducers likely to be the cause of the IFN-α production in SLE and have the significant advantage of targeting the NIPCs/PDCs selectively, without affecting their viability. The major questions here is obviously whether inhibition of the type I IFN system in SLE patients actually is of any benefit and whether injections of (humanized) anti–BDCA-2 antibodies have a therapeutic effect in SLE. Obviously, this issue can only be resolved by studies in patients and it might be wise to actually begin with some agents closer to clinical trials, such as neutralizing anti–IFN-α antibodies (36) or soluble IFNAR.

**Inhibition of the Type I IFN System May Have Adverse Effects.** Before embarking on clinical trials with the objective to inhibit the type I IFN system in SLE patients, potential adverse effects should be considered. Increased susceptibility to viral infections might for instance be expected, because this is seen in mice with inactivated IFNAR (1). This may pose an even greater problem in SLE patients, who already have a propensity to develop infectious diseases that contribute to an increased mortality. Consequently, the therapeutic goal in SLE must be to selectively inhibit the disease-related overproduction of IFN-α and at the same time spare the capacity of the type I IFN system to defeat viral infections. For this reason it is urgent to clarify the significance of the NIPCs/PDCs in immunity, especially their IFN-α production. This is important, because inhibition of the latter by for instance anti–BDCA-2 treatment could well dramatically impair the innate immunity to viruses in the lymphoid organs, where NIPC/PDC-derived IFN-α mainly is produced. Furthermore, if BDCA-2 ligation of NIPCs/PDCs still allows their differentiation to mature DC2, the immune system might be skewed toward a Th2 response, resulting in for instance inefficient adaptive antiviral immunity and enhanced production of autoantibodies.

In this commentary we have focused on the role of the type I IFN system in SLE, but it may well be important also in the development of other forms of autoimmunity. As mentioned before, IFN-α treatment of patients with tumors or infections can induce a broad spectrum of autoimmune diseases, including autoimmune hepatitis, thyroiditis, polymyositis, thrombocytopenia, rheumatoid arthritis, and insulin-dependent diabetes mellitus (IDDM; reference 22). Besides SLE, the best characterized naturally occurring autoimmune disease connected to type I IFN is IDDM, where increased serum levels of IFN-α and expression of IFN-α in the pancreatic islets have been observed (37). Furthermore, in experimental diabetes, the expression of IFN-α genes in islets precedes lymphocyte infiltration and islet cell destruction, which can be prevented by neutralizing anti–IFN-α antibodies (38). Expression of IFN-β have also been demonstrated in joint tissue in human rheumatoid arthritis and suggested to be of etiopathogenic significance (2). Further studies of the connection between type I IFN and human autoimmune disease are therefore highly warranted.

In conclusion, type I IFNs can contribute to loss of tolerance and promote an autoimmune response to several autoantigens, causing autoimmune reactions in many different organs. The propensity of type I IFN to cause autoimmunity may be related to the many immunostimu-
latory effects by these cytokines, but the genetic predisposition in a certain individual will eventually determine the susceptibility to develop a specific autoimmune disease during the prolonged type I IFN system activation. The risk for such autoimmune reactions may be the price we have to pay to be equipped with an efficient defense system against microorganisms, especially viral infections. A great challenge for the future is to learn more about the type I IFN system in both health and disease and how this system can be controlled and therapeutically modulated.

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