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Dendritic Cells Under Investigation in Autoimmune Disease

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ABSTRACT: Autoimmune disorders play an increasing role in public health, especially in light of the fact of the growing aged population, which primarily develop such diseases. A clear understanding of the mechanisms leading to the development of autoimmune responses and finally to autoimmune disease does not exist. Autoimmunity is characterized by the presence of autoantibodies and/or autoreactive T cells and the corresponding organ manifestation. Following the discovery of autoreactive T cells found in the periphery of mice and humans, the old immunological concept that autoreactive T cells are completely deleted in the thymus during evolution has been revised in recent years. Although antigen-presenting cells and particularly dendritic cells are known to play an important role in the regulation of immune responses and the activation of T cells, recent evidence suggests that the role of dendritic cells in the development of autoimmunity has been underestimated previously. This article aims to give a general overview on the basic immunological principles involved and gives a short review of the current literature on the functional relevance of dendritic cells in various human and murine autoimmune disorders.

ABBREVIATIONS: AARDA: American Autoimmune Related Diseases Association; **APC**: antigen presenting cell; **CTL**: cytotoxic T lymphocyte; **DC**: dendritic cell; **EAE**: experimental autoimmune encephalomyelitis; **IDDM**: insulin-dependent diabetes mellitus; **MCTD**: mixed connective tissue disease; **MHC**: major histocompatibility complex; **MS**: multiple sclerosis; **NOD**: non-obese diabetic; **RA**: rheumatoid arthritis; **RIP**: rat insulin promoter; **SLE**; systemic lupus erythematosus; **TNF**: tumor necrosis factor.

I. INTRODUCTION

Autoimmune diseases play an ever increasing role in public health in Western countries, and according to the American Autoimmune Related Diseases Association (AARDA[1]) collectively affect over 20% of the U.S. population. Accordingly, the incidence of autoimmune disorders makes it one of the top five most prevalent diseases within North America.[1] Among the most well known are systemic lupus erythematosus (SLE), Hashimoto's autoimmune

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thyroditis, multiple sclerosis (MS), type 1 diabetes (insulin-dependent diabetes mellitus, IDDM), psoriasis, and rheumatoid arthritis (RA). Although rarely lethal, significant morbidity and disabilities are a result. The etiology and pathogenesis of these illnesses are diverse, and many of the mechanisms behind the manifestation of autoimmune disorders are unknown or remain controversial. This is in part due to the multifaceted aspects of the disease as both genetic predisposition and environmental triggers affect the immune status of the cells and contribute to the susceptibility to autoimmune responses. To date most of the data on the progression of autoimmunity in humans are restricted to processes after the outbreak of disease for the obvious reason that people do not tend to visit their doctors in hopes of being diagnosed for an autoimmune affliction. Various animal models of autoimmune disease (Table 1), in particular SLE (reviewed in Refs. 2 and 3), have now been established and are facilitating the dissection of the pathways leading to autoimmune responses at all stages of disease development.

II. PRESENCE OF AUTOREACTIVE T CELLS WITHOUT DISEASE

Three major models reflect how the immune system works: the so-called Self-Nonself model predicts that the immune system is based on its ability to discern between self and foreign constituents, thus allowing the subsequent destruction of foreign pathogens. The Infectious-Nonself model adds the aspect that the activation of antigen-presenting cells (APC), generally the first immune cell type to be involved in a primary immune response, via costimulation is a necessary component of the immune response. APCs are not antigen-specific and present all sorts of antigens. Therefore, the newest model is the Danger Model. This model proposes that danger signals supplied by tissues injured by trauma, pathogens, toxins, etc. activate the APCs possibly in a tissue-dependent manner.[4] In this context, the effector cells must be able to recognize the antigenic constituents along with the danger signals that mark the presence of an invading pathogen. Complex interactions of a vast array of cell types and cell-signaling processes along with the specific ontological development of two of the major cell types involved in the immune process, the T and B cells, are necessary to achieve this goal. During intrathymic development, somatic selection processes allow a fraction of the T cells to differentiate, mature, and to be released into the body. In this respect, autoantigens play an essential role in establishing the repertoire of mature T cells. In the first phase of T cell selection within the thymus termed "positive selection", T cells with a sufficient affinity to self-antigens are selected for survival, whereas the remaining T cells die by neglect via apoptosis. In the second phase, termed "negative selection", T cells with an excessive affinity to self-ligands are once again deleted from the system via the apoptotic pathway. The remaining T cells then constitute the T cell battery enabling the recognition and combat of foreign pathogens. Some are polyactive and can recognize self antigens as well as foreign antigens.[5,6] A similar mechanism for the selection of self-reactive B cell precursors within the bone marrow has been postulated, although this pathway has not been studied extensively. Yet under the conditions prevailing in the thymus, not all self-antigens are presented and T cells possessing high avidity to self antigens can slip through the selection process. In contrast to the longstanding dogma that autoreactive cells are completely removed from the sys-

TABLE 1
Animal Models of Autoimmune Disease

TRANSGENIC MODELS		
Gene/Promoter	Model for	Reference
BAFF (TALL-1, THANK; zTNF4, BlyS)/ human ApoE enhancer promoter	SLE	MacKay et al. 1999 (84)
bcl-2/immunoglobulin heavy chain enhancer (Eμ-bcl-2)	SLE	Strasser et al., 1991 (85); Lopez Hoyos et al.,1996 (86)
CD40L/ K14-promoter	Mixed connective tissue disease/SLE	Mehling et. al 2001 (42)
CD19	Systemic sclerosis	Zhou et al., 1994 (87); Sato et al., 2000 (88)
Bovine GH/ phosphoenolpyruvate carboxykinase promoter (PEPCK-bGH)	RA	Ogueta et al., 1997 (89)
GM-CSF/HK ATPase ß-subunit promoter	Autoimmune gastritis	Biondo et al. 2001 (62)
IFN gamma/involucrin promoter	SLE	Seery et al. 1997 (90)
huIL-1- α/CAG promoter	RA	Niki et al. 2001 (91)
IL-4/MHC class I promoter	SLE	Erb et al., 1997 (92)
IL-6/K14 promoter	Psoriasis-like	Turksen et al., 1992 (93)
LCMV/RIP-gp promotre	Diabetes	Ohashi et al., 1991 (94)
TALL-1 (BAFF, THANK; zTNF4, BlyS)/ ß actin promoter	SLE	Khare et al., 2000 (95)
TGF-α/K14-promoter	Psoriasis-like	Vassar and Fuchs 1991 (96)
TNF- α	RA	Keffer et al., 1991 (97)
TNF- α /RIP promoter	Diabetes	Green et al., 1998 (44)
zTNF4 (BAFF, THANK; TALL-1, BlyS)/ V_H-promoter	SLE	Gross et al., 2000 (98)
KNOCKOUT MODELS		
GADD45a$^{-/-}$	SLE	Salvador et al., 2002 (99)
Fc gamma RIIB$^{-/-}$	SLE	Bolland et al., 2002 (100)
Bim$^{-/-}$	SLE	Bouillet et al., 1999 (101)
IL-2$^{-/-}$	SLE	Horak et al., 1995 (102)
Lyn$^{-/-}$	SLE	Nishizumi 1995 (103); Hibbs 1995 (104)
PD-1$^{-/-}$	SLE	Nishimura et. al. 1999 (105)
TACI$^{-/-}$	SLE	Yan et al., 2001 (106)
TGF-beta1$^{-/-}$	SLE/Sjögren's Syndrom	Kulkarni et al., 1993 (107)
TTP$^{-/-}$ (Tristaprolin)	SLE	Taylor et al., 1996 (108)
MRL/Fas^{lpr}/ TNFR1$^{-/-}$	SLE	Zhou et al., 1996 (109)
Desmoglein 3$^{-/-}$	Pemphigus vulgaris	Koch et al., 1997 (123)
SPONTANEOUS MUTATION MODELS		
BXSB	SLE	DesJardin et al. 1996 (110)
Cpdm/cpdm	Chronic proliferative dermatitis	HogenEsch et al., 1993 (111)
"Flaky skin" (fsn)	Psoriasis	Pelsue et al., 1998 (112)
LG/J	SLE	Peng et al., 1996 (113)
motheaten *(me^v/me^v)*	SLE	Tsui et al. 1994 (114)
MRL-lpr/lpr	SLE	Wang et al., 1997 (115)
MRL/Mp+/+	SLE	Theofilopoulos et al. 1985 (116)
(NZB x NZW) and (NZB xSWR) F_1s	SLE	Rozzo et al., 1994 (117)
Palmerston North mice (PN/n)	SLE	Walker et. al, 1978 (118)
Tight skin (TSK1/+) mouse-1/ fbn-1	systemic sclerosis (SSc), scleroderma	Green et al., 1976 (119); Siracusa et al., 1996 (120)
Tight skin (TSK2/+) mouse-2	Sxstemic sclerosis (SSc), scleroderma	Peters & Ball, 1986 (121); Christner et al., 1995 (122)

$^{-/-}$ knockout; gene-targeted

This list is by no means exhaustive and many other mouse models for autoimmunity exist.

tem via clonal deletion, new studies have revealed that, in fact, autoreactivity is necessary for immune functions as low-grade exposure to autoantigens in the periphery seem to be necessary for T cell survival.[7] Because these T cells are per definition autoreactive,[8] it is also essential to differentiate between autoimmunity and autoimmune disease in this context.

III. DENDRITIC CELLS AS REGULATORS OF (AUTO)IMMUNE RESPONSES

Autoreactive T cells have been detected in the periphery and blood in both rodents and humans (reviewed in Refs. 9 and 10). These autoreactive T cells are normally not activated as effective checks and balances such as antigen presentation in the absence of costimulation are present to avoid inadvertent stimulation and the subsequent development of autoimmune disease. Although most autoimmune disorders are generally referred to as being T cell mediated, one question must be asked: Which mechanisms play a role in the activation and/or induction of autoreactive T cells leading to the breakdown of immunological self-tolerance? Because antigen-presenting cells (APC) are highly capable of activating T cells, they can logically be implicated in the activation of autoreactive T (and B) cells. This puts us onto the direct path to dendritic cells (DC), because they are the most effective APC known today.[11] DC are the unrivaled champions in antigen presentation. In the periphery, they build a network of sentinel cells that untiringly sample antigens. They are extremely adept in antigen uptake because they are equipped with the means to ingest antigens by a manifold of different mechanisms, for example, via pinocytosis, macropinocytosis, mannose receptor-mediated endocytosis, and phagocytosis. The antigenic material is then processed and loaded onto the MHC molecules. DC play a key role in antigen translocation, as antigen transport from periphery to lymph nodes is primarily achieved by DC. Intrinsic signals and extrinsic signals, such as trauma or infection, induce the release of various cytokines, such as tumor necrosis factor (TNF) alpha, in the periphery, which generates a "danger" signal.[4] After sensing these danger signals, DC undergo profound morphological and physiological changes and begin to migrate from the periphery to the lymph nodes. They simultaneously undergo a process called maturation in which they switch from the antigen uptake mode to an antigen presentation mode. The antigen presentation mode is characterized by the upregulation of the various costimulatory and adhesion molecules necessary for T cell stimulation along with an intracellular redistribution of MHC molecules, which results in an increase of antigen-laden MHC on the cell surface. After contact with the T cells, the very high density of MHC molecules on the cell surface at this stage make DC extremely well equipped to present antigens to T cells via antigen-specific TCR engagement. However, not only the TCR/MHC engagement is required for effective antigen presentation, but also costimulatory molecules need to be present in order to elicit an immune response. Among these costimulatory molecules one receptor/ligand pair plays a prominent role in this context and that is the CD40 receptor on DC and its ligand CD154 (CD40L) on T cells. Ligation of this receptor pair leads to activation signals both in DC as well as in T cells.[12] Furthermore, ligation of CD40 provides one of the strongest activation signals for DC known today and results in the production of IL-12 and the skewing of T cell responses toward the Th1 type.[13] As CD40/CD154 interaction is also crucial for B cell stimula-

tion and immunoglobulin class switching, this suggests that CD40 engagement plays an essential role in the communication between DC, T, and B cells. Therefore, it has been proposed that CD40/CD40L cross-linking contributes to the generation of autoimmune responses. DC can prime both $CD4^+$ or $CD8^+$ T cells and B cells independently of each other.[14,15] Consequently, they not only play a role in initiating cytotoxic immune responses but also play a role in the regulation of humoral responses.

On the other hand, DC also play a contrasting and yet pivotal role in promoting tolerance. During T cell development, thymic DC are involved in the processes for deleting autoreactive T cells.[16] In addition, evidence is growing that in particular DC of the immature phenotype are responsible for downregulating immune responses. Although mature DC do not seem to be affected by interleukin(IL)-10,[17] the presence of IL-10, a Th2-promoting cytokine, during the initial stages of activation of DC causes a reduction in the maturation process of DC and can induce tolerance.[18,19] This includes the downregulation of inflammatory cytokines such as IL-6, IL-1ß, and TNFα along with a downregulation of MHC class II molecules and various costimulatory and adhesion factors. Jonuleit et al. reported that repeated stimulation of T cells, with immature DC results in the generation of regulatory IL-10 producing $CD4^+$ $CD25^+$ T cells which in turn promote tolerogenicity.[20] Interestingly, two reports in which hemaglutinin or ovalbumin function as model self-antigens of the periphery revealed that the presentation of autoantigens via DC are a prerequisite for CD4- and CD8-mediated tolerance.[21,22] In mice, DC that were generated in the presence of TNF-α and pulsed with autoantigenic peptide ameliorated experimental autoimmune encephalomyelitis already after the first injection.[23] TNF-α-treated DC expressed MHC class II as well as costimulatory molecules, but produced significantly higher concentrations of IL-10 compared with CD40L-treated DC. In two volunteers, the injection of immature DC pulsed with influenza matrix peptide (MP) led to a specific inhibition of MP-specific $CD8^+$ T cell effector function.[24] Recently, it was demonstrated by the same investigators that application of immature DC-induced hapten-specific CD8+ regulatory T cells *in vivo*.[25] These data suggest that immature DC might be a useful tool for the therapeutic down-regulation of antigen-specific immunity.

In the case of autoimmunity, players in the immune system go astray, the most well known being dysregulated T cells and B cells. Normally, these cells are held in abeyance by the simple lack of the costimulatory molecules in the microenvironment necessary to stimulate them.[26] T cells and in particular $CD8^+$ but also $CD4^+$ T cells are the effectors cells that attack or activate target cells and elicit tissue destruction in this scenario (Figure 1). B cells produce large quantities of antibodies, and various mechanisms can lead to tissue injury, for example, by the formation of large deposits of immune complexes in the kidney often leads to nephritis and tissue damage. Furthermore, autoantibodies can initiate cytolysis of the cells or opsonize them for elimination via phagocytosis. Activated B cells also play another role in the elicitation of autoimmune responses, namely, they themselves can function as antigen-presenting cells that present autoantigens to the relevant T cells and contribute to epitope spreading. In the last few years evidence has accumulated that DC play a much larger role in autoimmune responses than originally thought. DC are accomplices in the elicitation of autoimmunity in that they play a pivotal role in the activation of both T cells and B cells and by delivering the autoantigens with the appropriate factors necessary to elicit an immune

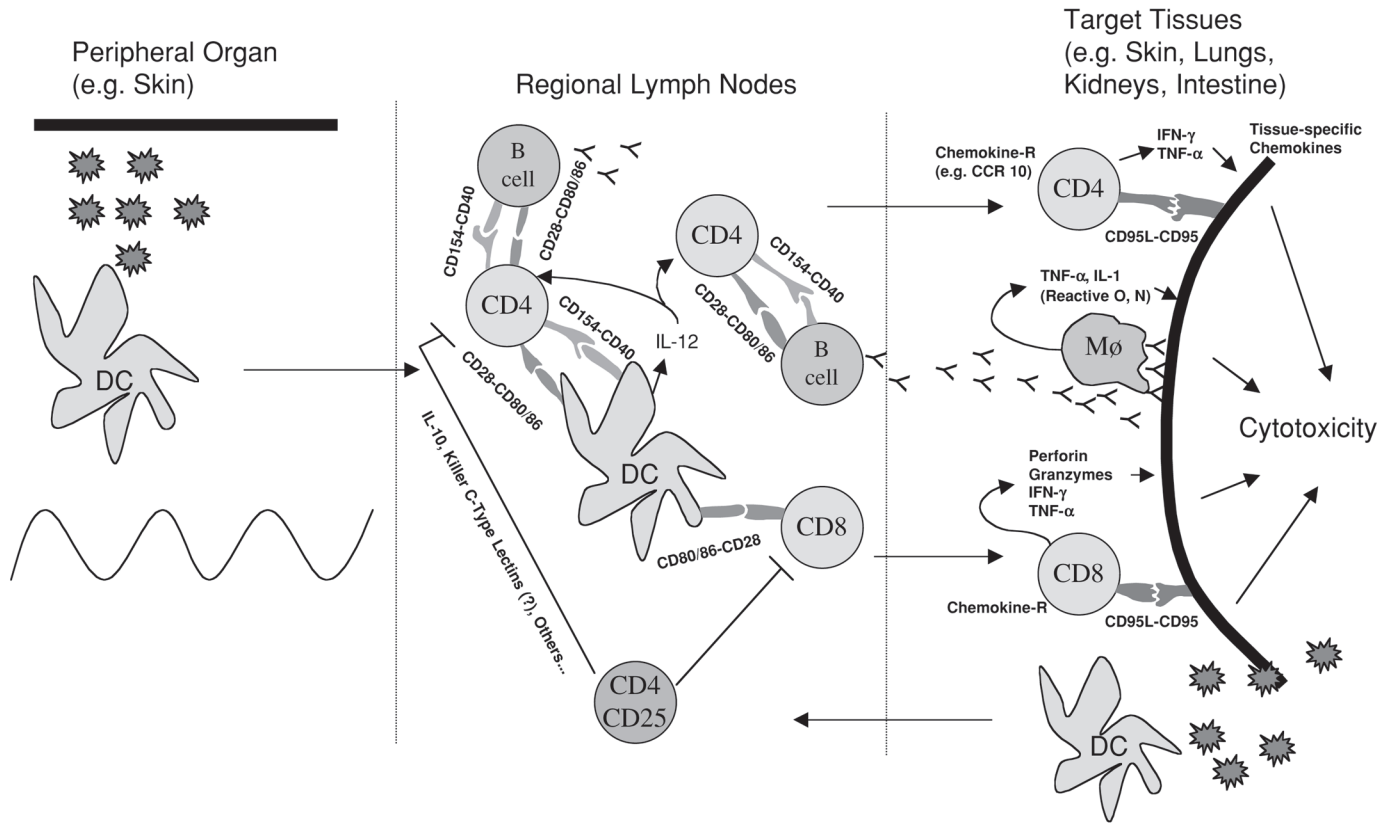

FIGURE 1. A role for dendritic cells in the induction of autoimmune responses and autoimmune disease. (A color version of this figure is available in the on-line copy of the article.)

response (Figure 1). Because DC cannot discriminate between the antigens they capture at the site of inflammation and thus will inadvertently present self-antigens along with pathogen-specific antigens, a complex interplay of factors has evolved as a necessity for the prerequisite maturation of DC needed for the activation of naive T cells. DC react differently to dying cells as necrotic cells elicit other responses than apoptotic cells from DC.[27] DC promote stimulatory responses from $CD4^+$ and $CD8^+$ T cells following exposure to necrotic cells (cell death due to trauma or infection), whereas DC that have had contact to apoptotic cells (physiologic cell death) do not.

An emerging factor in the induction of autoimmune disease is the uptake and processing of autoantigens originating from apoptotic cells. This model has been receiving increased attention as the latest evidence seems to support the idea that the self-determinants involved in autoimmune responses are supplied by the dying cells as the reservoir for autoantigens. Physiologic apoptosis is generally considered to promote tolerogenicity and not autoimmunity. Apoptosis usually occurs sporadically and asynchronously within tissues, with no proinflammatory cytokines present. Under steady state conditions, T cells presented with autoantigens by DC either become anergic or are deleted. Yet, during the apoptotic process, many proteins and other cell constituents are uniquely modified that can expose cryptic epitopes or even generate novel autoantigens.[28] Antiphospholipid antibodies are found in some patients afflicted with SLE. Phosphatidyl-serine is generally located on the inner surface of the plasma membrane, yet it is exposed to the outside on the surface of apoptotic blebs, thus making it accessible as an epitope.[29] Other modifications are the apoptosis-specific proteolytic cleavage and/or phosphorylation of the substrate molecules. Defects in apoptotic cell clearance also seem to play a central role. Macrophages and DC scavenge apoptotic cells, ingest these cell components, and present them to T cells via MHC complexes, thus initiating the signaling cascade leading to autoimmune disease. Furthermore, increased or aberrant apoptotic rates have been reported in tissue-specific as well as systemic autoimmune disorders. At this point it is important to note that exposure to high numbers of apoptotic cells is able to mature DC even in the absence of further inflammatory signals,[30] and this may explain why the threshold for an autoimmune response is reached.

This is especially interesting when microbial insult leads to the generation of an immune response to such antigens. Emerging evidence also points to a role of pathogens in the modulation of DC function. The infectious agents associated with autoimmune disease are diverse and include the involvement of streptococci in rheumatic fever, Borrelia burgdorfii in Lyme arthritis, Coxsackie virus in myocarditis, Cytomegalie virus or rubella in type 1 diabetes, etc.[31] Although only circumstantial evidence is yet available and the underlying mechanisms as to how they set off disease are unclear, it intriguingly incriminates APC in this context. One possibility is that APC present self-determinants, a simple bystander activation process takes place and nonspecifically activates the cells of the immune system in the close vicinity or within the lymph node during the immune response to the microbes. The process of "T cell epitope mimicry" has also been proposed to be a major factor in the proclivity of microbial infection to elicit processes leading to autoimmune diseases.[31] In this case, as antigen recognition/specificity by T cells can be degenerate, T cells can recognize autoantigens that are similar to those of the pathogen. A recent report by Ludewig et al.

revealed that stringent threshold levels not only of antigen load but also of the duration of (auto)antigen presentation determines whether stimulation of self-reactive CTLs with subsequent autoimmunity ensues or not.[32] In this context, the maturation status of the DC is of prime importance: immature DC have a high turnover rate of MHC/antigen complexes on their cell surface, whereas mature DC downregulate turnover rates and stabilize the presence of the antigen/MHC complex on the surface of the DC.[33]

IV. DC IN VARIOUS AUTOIMMUNE DISORDERS

In the following, the role of DC in various autoimmune diseases is described. They indicate that local or recruited DC play a fundamental role in the effector phase of these diseases even before the overt phase of disease is reached. The early infiltration into tissues and the abnormal accumulation of APC prior to the migration of macrophages, B, and T cells into these areas indicate that signaling from microenvironment and activation of DC pave the way for disease progression. Danger signals probably originate from necrotic cells following injury or microbial insult. Focal neogenesis of lymphoid tissues can ensue as the disease advances. Within these tissues, DC interact with the other immunocompetent cells and thus contribute to the affinity maturation of B cells, the production of autoantibodies, and the stimulation and expansion of T cells. The distorted homeostatic balance between the cells in the autoimmune diseased areas results in the maintenance of the leukocytic infiltrate and a persisting inflammatory process.

A. Systemic Lupus Erythematosus (SLE)

SLE is one of the prototypic systemic autoimmune diseases and various mouse models with characteristics of the disease are available for study. Among the various symptoms of this disease, SLE is characterized by B cell hyperactivity, renal immune complex depositions and resulting nephritis, anti-dsDNA autoantibodies along with depositions. In recent studies, a connection to the CD40/CD154 dyad and to enhanced migration of DC to the spleen have been found. Within the serum of patients suffering from SLE, increased concentrations of soluble (s)CD40L were detected and, interestingly, these serum concentrations correlated with disease activity. Also, the expression of CD40L was found to be up-regulated on T cells from patients with SLE.[34] Scheinecker et al. reported clinical correlations between DC numbers within the blood and SLE.[35] Patients with SLE exhibited profound changes in functional and phenotypical parameters of the DC and had an average of 80% fewer CD11c-positive DC. Using the mouse model of SLE (SWR x NZB)F(1) (SNF(1)) mice, both Lettesjo et al. and Kalled et al. found that disease is dependent on CD40/CD154 interactions, and that there was an increase in splenic cell numbers of DC.[36,37] This group further postulated that this was due to an enhanced migration of DC from the periphery to the spleen and dysregulated DC homeostasis. Disrupted DC homeostasis was also proposed to be key to SLE pathogenesis by Blanco and co-workers.[38] Monocytes isolated from the blood of patients suffering from SLE were potent activators of T cells *in vitro*, indicating that they were actually differentiated DC. Furthermore, normal monocytes were induced to differentiate and to develop DC-like morphology and function after stimulation

with serum from SLE patients. The active agent was identified as being IFN-α because only antibodies directed against this molecule could abrogate the stimulating activity of the serum.

B. Mixed Connective Tissue Disease (MCTD)

MCTD is an autoimmune overlap syndrome that includes aspects of lupus erythematosus, scleroderma, and/or dermatomyositis.[39-41] Studies in our laboratory aimed at investigating the role of chronically activated tissue-resident DC of the epidermis, the Langerhans cells, in the generation of autoimmune responses. Transgenic mice were generated that overexpress the strongest stimulator of DC, CD40L, in Langerhans cell neighboring basal keratinocytes.[42] Our results obtained from these Keratin14-CD40L transgenic mice further substantiate the role of DC in the elicitation of autoimmunity. In this model mouse system many of the quintessential aspects of MCTD, especially with respect to lupus/lupus-like manifestations, were generated, including substantial inflammatory autoimmune scleroderma-like skin lesions. Similar to the study mentioned above, these mice were found to have drastically decreased numbers of epidermal Langerhans cells and increased numbers of activated DC within the dermis. This was especially evident in the affected skin areas. Moreover, these effects were accompanied by a concurrent increase in DC cell numbers in the lymph nodes draining from areas of inflammation and in the spleen. DC migration to the lymph nodes also seemed to be enhanced, suggesting that an increased migration of CD40-activated DC from the periphery was taking place. Serum analyses revealed the presence of antinuclear as well as anti-dsDNA antibodies. These autoantibodies were directed against the skin as indicated in indirect immunofluorescence stainings. Moreover, autoantibody depositions were found at the dermo-epidermal junction and at the glomeruli of the kidneys, which led to the loss of renal function and proteinuria. Additionally, pulmonary as well as esophageal fibrosis were also present in these animals. Together, the data indicate the development of a systemic autoimmune disease in Keratin14-CD40L transgenic mice with striking aspects of both scleroderma and systemic lupus erythematosus. Unexpectedly, the inflammatory autoimmune phenotype also developed in B-cell-deficient mice Keratin14-CD40L transgenic double mutants. Interestingly, T cell transfer experiments with $CD8^+$, but not $CD4^+$, T cells from transgenic mice elicited the manifestation of the autoimmune disease in the recipient animals, suggesting the presence of autoreactive T cells. In this system, these pathologies occurred spontaneously without the need for immunizations with specific antigens, antigen-pulsed DC, antigen-specific T cell transfer, microbial infection, etc. In this context, it is interesting to note that DC themselves can activate T cells, circumventing the necessity for B cells in this process. This makes this mouse model an excellent system in which to dissect in detail the contributions of various cell types and pathways, in particular cellular (T cell) vs. humoral (B cell)-dependent immunological mechanisms, leading to the generation of autoimmunity and finally to the exacerbation of a systemic autoimmune disease. These results point to an inherent role of aberrant activated DC, and in this study the novel finding of LC in particular in breaking tolerance against self-antigens resulting in the development not only of cutaneous, but surprisingly also of widespread systemic autoimmunity. Further studies will determine through which mechanisms Langer-

hans cells are chronically activated *in situ* in human skin. Knowing that increased apoptosis can result in the activation of DC, aberrant and prolonged apoptotic rates have been reported in keratinocytes within ultraviolet-induced lesions of cutaneous lupus. Another intriguing possibility would be the expression of CD40L on skin mast cells, which has not been investigated so far. Our findings in turn raise the question whether the postulated role of B cells and their antibody production are the causal elicitators of lupus-like syndromes or only a secondary effect following aberrant DC activation. In this model and taken together with the data accumulated by Garza et al., clear evidence is given that a crucial aspect of tolerance vs. autoimmunity is determined by the activation state of the DC.[43]

C. Diabetes Mellitus

In a mouse model of diabetes, dendritic cells were also reported to be of critical importance in the pathogenesis of islet autoimmunity in nonobese diabetic (NOD) mice. Green et al. reported that organ-specific autoimmunity is triggered by antigen-presenting cells in transgenic mice that overexpress TNF-alpha in the insulin secreting β-cells of the islets of Langerhans.[44] TNF-alpha is an inflammatory molecule that is expressed by numerous different cell types such as activated $CD4^+$ T cells, macrophages, and keratinocytes. By placing the gene for TNF-alpha under the control of the rat insulin promoter (RIP), accelerated progression of autoimmune diabetes in neonatal NOD mice was achieved. Disease was preceded by localized apoptosis of some islet cells, the upregulation of MHC class I on the residual islet cells, and by the sequential infiltration of DC and macrophages, B cells, $CD4^+$, and $CD8^+$ T cells into the islets. Freshly prepared DC and macrophages but not B cells were in turn able to present MHC/islet-peptide complexes to $CD4^+$ T cells *in vivo* leading to activation of these cells. As mentioned previously, TNF-alpha is a "danger" signal for DC, and accordingly upregulation of activation markers on DC as well as on T cells infiltrating the islets was observed. CD40/CD40L interactions on mucosal DC was also implicated in contributing to the etiology of diabetes.[45,46] These data give considerable support to the concept of DC activated by inflammatory processes mediating autoimmune disease by the presentation of (auto)antigens and subsequent stimulation of autoreactive T cells.

D. Rheumatoid Arthritis (RA)

DC are believed to have a critical role in the pathogenesis of RA. An early infiltration of differentiated DC into the synovial tissue prior to the exacerbation of disease has been observed.[47] DC are found in high numbers in the serum and synovial fluids of patient afflicted with RA.[48,49] Analysis of tissue biopsies from patients with RA revealed a close association of DC and T cells within inflammatory sites.[50] These data point to signals present within these inflammatory sites that lead to the accumulation of the DC and T cells within these areas. Secondary lymphoid follicle formation in areas of inflammation along with a strong germinal center reaction has been observed.[51,52] In synovial tissues of patients with RA follicular dendritic cells are in an activated state and extensive DC/T cell interactions take place. Thomas et al. reported that the circulating DC in patients with RA are identical to those of normal individuals.[53] In contrast, fully differentiated DC associate with T cells, and the rheumatoid tissue represents an effector site reminiscent of a delayed-type hypersensitivity responses. This group also

argues that the presentation of autoantigens by DC is a major factor in the continual autoimmune response.

E. Multiple Sclerosis (MS)

In humans, high levels of activated DC are associated with MS. These DC secrete proinflammatory cytokines that may contribute to the induction and maintenance of disease.[54] Presentation of myelin basic protein by DC has also been implicated in the initiation of experimental autoimmune encephalomyelitis (EAE), the animal model for the human disease multiple sclerosis. EAE in this model system is induced by the injection of proteins derived from the myelin sheath, for example, myelin basic protein (MBP). In a novel method, the acute monophasic disease course was induced by the injection of DC pulsed with the $A_{c\ 1\text{-}11}$ MBP, a peptide recognized by T cells that can elicit EAE.[55] The underlying molecular mechanisms may involve cryptic epitopes and determinant spreading leading to a diversification of the immune response.[56] It has been postulated that $CD4^+$ T cells are effectively activated by DC in disease development, and that the involvement of B cells in the activation of the T cells as well as in epitope spreading in this model system does not play a fundamental role.[57]

F. Sjögren's Syndrome

Sjögren's syndrome is a further autoimmune disorder in which DC play an essential role in the course of the illness. This disease is characterized by lymphocytic infiltrates in the salivary and lacrimal glands. In a recent study, van Blokland et al. used the NOD and MRL/lpr mouse models to particularly focus on APC in the pathogenic process.[58] Increased numbers of DC were detected in the submandibular glands of NOD/SCID mice prior to lymphocytic infiltration and the outbreak of sialoadenitis in these mice. The same group found correlating results in the human counterparts.[59] A differentiation was made between patients suffering from Sjögren's syndrome and those with focal sialoadenitis without the clinical criteria of Sjögren's syndrome.

G. Gastrointestinal Autoimmunity

There is also evidence that activated DC play a role in autoimmune diseases of the gastric/intestinal tract. The results of a recent study by Ikeda et al. also point to an essential role of activated DC in the exacerbation for ulcerative colitis in humans.[60] $CD83^+$ DC were not only found in the inflamed intestinal mucosa of patients suffering from Crohn's disease or ulcerative colitis but also in the peripheral blood of these patients. Vukovic et al. also found activated CD40+/CD86+ DC in the inflammatory tissue of patients with inflammatory bowl disease pointing to an aberrant regulation of DC.[61] In the animal model of autoimmune gastritis, Biondo et al. targeted GM-CSF overexpression to the gastric mucosa.[62] Once again, an influx of DC in early stages of disease was observed. DC were implicated in the uptake of apoptotic cells resulting from the turnover of the cells of the stomach and the activation of naïve CD4 T cells specific for the autoantigen HK ATPase. Leithauser et al. used eGFP-expressing $CD4^+$ T cells to track the repopulation of intestinal tract and the lymph nodes of RAG1(–/–) mice injected with these donor cells.[63] DC aggregates in which the CD4 T cells clustered and proliferated were identified in the mucosa/submucosa prior to manifestation

of disease once again implicating DC in disease development. In a recent study, a new model of murine colitis was described. In this model, the transfer of $CD45RB^{hi}CD4^{+}$ T cells into immune-deficient mice resulted in intestinal inflammation. The development of colitis was reported to be dependent on the accumulation of $CD134L^{+}$ (OX40L) DC within the mesenteric lymph nodes. Moreover, CD134L was expressed on DC in SCID mice in the absence of T cell signaling. Colitis did not develop when $CD4^{+}CD25^{+}$ regulatory T cells were present, which points to a possible role for regulatory T cells in controlling the DC activation process and preventing DC-mediated immune pathology.[64]

V. CONCLUSIONS AND THERAPEUTIC OUTLOOK

Aberrant DC function has also been implicated in other, less prevalent autoimmune diseases such as Wiskott-Aldrich syndrome, ALPS II, and sarcoidosis.[65-67] The various autoimmune diseases described indicate that local or recruited DC play a fundamental role in the effector phase of these disorders even before the overt phase of illness is reached.

Women are more affected than men by autoimmune disease. Lupus, along with Sjögren's sydrome, Hashimoto's autoimmune thyroiditis, and scleroderma is known to predominantly affect women, since over 80% of the patients afflicted with these diseases are female.[68] Of the patient population suffering from RA, MS, and myasthenia gravis, 60 to 70% are female. Although there are very few reports on hormone effects on DC, circumstantial evidence could be interpreted to the means that DC are affected by female hormones. Because hyperprolactinemia is associated with autoimmune diseases, Matera et al. studied the effects of prolactin on DC.[69] This group was able to show that high doses of prolactin led to the maturation of DC and ergo possibly to an enhanced presentation of self-antigen resulting in autoimmune disease. On the other hand, the treatment of DC with nonsteroidal antiestrogens is able to inhibit the terminal maturation of DC.[70] Tamoxifen has been reported to be beneficial in the treatment of various experimental models of autoimmune diseases such as SLE and collagen-induced arthritis.[71-73] Recently, Komi et al. investigated the effects of tamoxifen and toremifene on DC.[70,74] As these substances had an inhibitory effect on DC maturation and function, a novel target of these drugs and new therapeutic potentials for the treatment of autoimmune diseases was revealed.

Although targeting T cells in immunomodulating drug therapy is effective, one thing is sure: There is communication between T cells, B cells, and DC, and it leads to the perpetuation of the disease. Since the CD40/CD40L pathway participates in the cell-cell activation of DC/T and DC/B cells and BC/T cells, it would provide an ideal target for therapeutic intervention. Indeed, animal studies have demonstrated that the functional interruption of CD40/CD40L signaling by the application of neutralizing antibodies leads to enhanced renal function with reduced proteinuria and nephritis in mice with SLE.[75] First human studies have also revealed a beneficial effect for CD40/CD40L blockade in decreasing IgG and IgG anti-DNA antibody-producing B cells found in patients afflicted with lupus.[76] Although we cannot answer the question to 100% as to what extent DC play the elicitating role in autoimmune disease, much evidence is at hand to implicate that these cells do play a larger role than originally thought and to disclose the pathologic potential of the DC. As the results of various studies also reveal, a therapeutic promise does lie in targeting

DC with immunotherapeutic drugs. A therapeutic agent used in transplantation medicine but also shown to ameliorate the symptoms found in cutaneous autoimmune diseases, including SLE, is the immunosuppressant mycophenolate mofetil (MMF). Work by our group revealed for the first time that MMF not only has an suppressive effect on T cells but also inhibits the maturation of DC, further pointing to a role of DC in the pathogenesis of SLE.[77] Glucocorticoids also suppress DC maturation, and these substances have also been used successfully in treating autoimmune diseases.[78] Vitamin D (1alpha,25-Dihydroxyvitamin D[3]) and some of its analogs has immunosuppressive effects resulting in part from the inhibitory effects it has on the differentiation and maturation of DC.[79-82] Recently, Huismann et al.[83] reported that women suffering from SLE or fibromyalgia have reduced levels of 1,25(OH)2-Vitamin D, possibly due to the use of hydroxychloroquine — is there a connection? Admittedly, this is very farfetched, but seeing things from a different viewpoint can give us a better vantage point and one can never know what results and therapeutic alternatives will ensue. Although the idea of immunosuppression via DC is also somewhat simplistic, and one must take into account that there is a delicate balance between immunity and autoimmunity, future therapeutic regimes used to combat autoimmune disease should not take DC lightly and may actually be combating autoimmune disease at its roots.

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