New Concepts on the Pathogenesis of Autoimmune Disease

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According to Burnet's clonal selection theory, autoreactive lymphocytes are deleted during their differentiation in the thymus. However, the negative selection process is not absolute, and self-reactive lymphocytes and antibodies are regularly found in healthy individuals. The persistence of autoreactive cells within the natural lymphocyte repertoire in many different species suggests that autoimmunity plays important roles in body homeostasis. In fact, natural autoantibodies were shown to be involved in immunoregulatory circuits through idiotypic interactions, to contribute to the defenses against pathogens, and to help in the clearance of altered self-constituents [1]. Moreover, Moalem et al. [2] recently provided evidence that autoimmune T lymphocytes could also be involved in the body maintenance by promoting the healing of damaged tissues, especially in the central nervous system.

Autoimmunity becomes pathogenic when the autoimmune response is persistent and results in the emergence of uncontrolled autoagressive effector cells. The development of a full-blown autoimmune disease will further require the penetration of the effector cells in the target organ, which might depend on local microvessel changes induced by inflammatory signals. Both genetic and environmental factors govern the nature of the autoimmune responses via multiple mechanisms. Indeed, both types of factors influence the presentation of major histocompatibility complex-restricted self- or self-mimicking epitopes to T cells in lymph nodes and target tissues, polarization of the T cell response (i.e., Th1-type versus Th2-type), emergence of regulatory cells, as well as the permissive status of the target organ to infiltration by immune cells.

Generation of autoaggressive T cells

Although it is clear that autoreactive T cells are present in the normal individual, these T cells normally do not induce pathology. To become pathological, the self-reactive T cells have to be activated to expand and to become effector cells. Effector T lymphocytes can induce self-tissue damage by helping in the generation of pathogenic autoantibodies or helping in the development of cellular immune responses mediated by macrophages and cytotoxic T lymphocytes. A first important regulatory point in the pathogenesis of autoimmune disease will thus be the regulation of the activation of self-reactive T cells. In this regard, dendritic cells probably play a crucial role.

DC are extremely specialized antigen-presenting cells that – according to their ontogeny and/or their mode of activation – can induce tolerance or specific immunity [3]. Thus, for example, in the presence of interleukin-10, monocyte-derived human DC do not produce IL-12 [4] and can anergize T cells [5]. Recent observations suggest that immature DC might also induce regulatory T cells. Moreover, some DC might induce the death of autoreactive T lymphocytes. In mice this property has been attributed to lymphoid DC through their expression of the Fas ligand molecule [6]. In humans, monocyte-derived DC activated during viral infection can kill sensitive lymphocyte targets through the TRAIL ligand [7], and we recently observed that even at the resting state they can kill Jurkatt T cells through an as yet unidentified caspase-dependent mechanism (Vanderheyde et al, manuscript submitted). Whatever the mechanism DC use, very elegant experiments *in vivo* have now clearly demonstrated that APC, and in particular DC, are necessary and sufficient to induce tolerance to peripheral antigens [8].

Some environmental or genetic factors might interfere with the tolerizing properties of DC and thereby promote a priming presentation of the antigen. Microbes and pro-inflammatory cytokines do indeed induce the maturation of DC, a process characterized by an up-regulation of co-stimulatory molecules necessary to optimally activate naive T cells, a modification of DC expression of the chemokine receptor allowing DC to migrate to the lymph nodes, and a stimulation of cytokine production. In particular, some microbes or microbial products such as lipopolysaccharide are potent stimulators of IL-12 production by DC [9,10]. In this regard, it is interesting to note that DC can respond to LPS only in the presence of the soluble form of CD14, a factor present in the serum [9]. Thus, increased vascular permeability associated with inflammation is likely to enhance DC activation by microbial products that require CD14 to do so.

Considerable experimental data support the concept that activated DC can induce autoimmune diseases. Indeed, DC pulsed with myelin basic protein elicited experimental allergic

DC = dendritic cells
IL = interleukin
APC = antigen-presenting cells
LPS = lipopolysaccharide

encephalomyelitis [11], DC pulsed with thyroglobulin triggered autoimmune thyroiditis [12], and vaccination against tumor using DC pulsed with tumor antigens shared with normal host resulted in severe autoimmune disease [13].

The epidemiological evidence of an association between some infections and autoimmune disease might be the consequence of what is called molecular mimicry but would also reflect the particular adjuvant effect of some pathogens. The theory of molecular mimicry holds that the autoimmune response results from similar epitopes shared by the pathogen and the host. Thus the immune response elicited against the pathogen will eliminate it, but because of similarity between pathogen antigens and some host antigens it will lead also to lesions of self-tissues. Many observations support this concept, such as the case of rheumatoid fever where patients have antibodies directed against a streptococcal protein envelope that cross-react with myosin, a myocardial protein, which eventually results in cardiac damage. Multiple sclerosis is another example of molecular mimicry involving autoreactive T cells. Indeed, T cell clones have been isolated from patients with MS that can cross-react with a variety of infectious agents. The involvement of microbes in the pathogenesis of autoimmune disease through their adjuvant effect is best illustrated by the work of Segal et al. [14,15]. These authors studied an animal model of MS – the experimental allergic encephalomyelitis – and showed that preexisting autoreactive T cells stay in a quiescent state unless microbial products such as lipopolysaccharide or CpG oligonucleotides are present. They also showed that such microbial factors are necessary to allow the development of effector Th1 cells and the induction of EAE through the stimulation of IL-12 production. The influence of the environment on the regulation of IL-12 production might therefore be critical for the development of Th1-mediated autoimmune disease in genetically susceptible individuals. The importance of IL-12 in the tolerance process is also illustrated by transplantation experiments in newborns. Newborn mice tolerated allogenic skin graft, and this tolerance can be abolished through the administration of IL-12 [16]. This observation as well as observations on human neonatal DC [17] suggest that the defect of IL-12 production by neonates is associated with a higher level of graft acceptance.

Infection could also favor the development of autoimmune disease by increasing the availability of self-antigens and by allowing their presentation. Normally self-reactive naive lymphocytes do not encounter their specific antigen and thus cannot expand in response to it, since naive lymphocytes circulate in blood and the secondary lymphoid organs but do not have access to peripheral tissues. The proposal made by different groups [18,19] is that infection would lead to the migration of APC-bearing self-antigens to draining lymph nodes. This would permit the activation of autoreactive T cells that, as effector cells, will be able to invade and destroy peripheral tissue. Infection can lead to this scenario by inducing tissue damage, leading to the liberation of autoantigens. These autoantigens can then be picked up by APC, and the inflammatory response induced by the microbe leads to the activation and migration of APC to draining lymph nodes. This would result in activation of self-reactive or cross-reactive T lymphocytes.

The availability of self-antigens depends also on the control of the clearance of apoptotic cells. Indeed, common autoantigens targeted in systemic lupus erythematosus are present at high density on the surface of apoptotic cells [20] and injection of apoptotic cells induces autoantibodies formation although not full-blown SLE [21]. The high predisposition for lupus-like syndromes of patients or mice deficient in some complement factors (particularly C1q and C4) may be explained by their impaired capability to clear apoptotic cells [22]. By opsonizing apoptotic cells, complement factors promote apoptotic cell clearance. Additional evidence supporting the importance of the clearance of apoptotic cells and their byproducts, such as chromatin, is inferred from the observation of mice deficient in serum amyloid protein [23]. Such mice develop a lupus-like syndrome, which can be explained by a defect in chromatin clearance in the absence of serum amyloid protein. Thus, when apoptotic cells accumulate, autoantigens that are normally cleared persist and could be presented by DC. DC can indeed phagocyte apoptotic cells and cross-present derived peptides whereas macrophages are unable to do so [24]. However, normally, apoptotic cells do not induce DC maturation and thus apoptotic cell-derived peptides would be presented in a neutral or tolerogenic fashion [25]. Additional factors such as pro-inflammatory cytokines or microbial products should thus be present to induce DC maturation for activation of autoreactive T cells. Apoptotic cells were initially described as anti-inflammatory since they inhibit the liberation of pro-inflammatory molecules by macrophages [26]. However, some forms of apoptosis can be inflammatory and could induce DC maturation [27], especially when apoptotic cells are coated with antibodies [26]. There is also evidence that post-apoptotic cells can be pro-inflammatory [28] as are necrotic cells [25]. In summary, if apoptosis occurs in "silence," presentation of self-peptides is likely to result in tolerance, whereas when apoptotic cell death takes place in a context of inflammation, in the presence of autoantibodies or in association with secondary necrosis (because of an overwhelming level of apoptosis or because of a defect in the clearance), then immunogenic presentation of self-peptides is likely to occur, resulting in autoimmunity.

Elimination of autoaggressive T cells

Elimination of T lymphocytes after their activation is essential both to allow the immune system to return to its basal state after an infection episode as well as for the control of

MS = multiple sclerosis
EAE = experimental allergic encephalomyelitis

SLE = systemic lupus erythematosus

autoimmunity [reviewed in 29]. This is best illustrated by the autoimmune phenotype of knock-out mice for the Fas or Fas-ligand genes that encode critical molecules for activation-induced cell death of T cells. Likewise, mutation of the Fas gene in humans is associated with an autoimmune lymphoproliferative syndrome [30]. On the other hand, enhancement of AICD might protect against autoimmune disease. Zhou et al. [30] demonstrated prevention of two experimental T cell-mediated autoimmune diseases in rats by enhancing T cell AICD using bisindolylmaleimide VIII [31]. Autoimmune disease might thus result from an imbalance between the proliferation of autoimmune T cells and their elimination by AICD. Things are not so simple however, since excessive AICD of T lymphocytes could also promote autoimmune pathology, as reported by different groups [32]. In this case, autoimmune pathology might result from a defect in the handling of apoptotic cells as we will discuss below.

Regulation of the fate of antigen-presenting cells is a less studied phenomenon but is probably as important for the control of autoimmunity. *In vivo* experiments in mice showed that the interactions between antigen-bearing dendritic cells and specific T lymphocytes are followed by DC disappearance [33,34]. Moreover, a mutation of an enzyme involved in the apoptosis program, caspase 10, underlies an autoimmune lymphoproliferative syndrome and is associated with a defect of DC apoptosis and accumulation of DC in lymphoid organs [35]. According to our observation that bisindolylmaleimide promotes death receptor-mediated apoptosis [10], the protective effect of this compound against autoimmune pathology might be due not only to its effects on T cell AICD but also to its ability to promote DC apoptosis.

Regulation of autoaggressive T cell function

Rodents that are either congenitally lymphopenic, or are rendered so, develop a number of organ-specific autoimmune diseases, and the correction of the lymphopenia by CD4 + T cells from normal donors prevents the development of the disease [reviewed in 36]. In humans also, induced lymphopenia has been associated with the development of autoimmune thyroid disease [37,38]. These observations reflect the importance of a subset of T lymphocytes that control the activation/function of autoreactive T cells normally present in all individuals. Such lymphocytes are termed regulatory T cells and probably represent a heterogenous population. One well-characterized subset is defined by its constitutive expression of the chain of IL-2 receptor (CD25), and its regulatory property seems to be linked to the constitutive expression of the CTLA-4 molecules (the alternative counter-receptor for B7 molecules). Another subset is termed Tr1 and is characterized by the production of high levels of IL-10, moderate amounts of transforming growth factor beta, interferon gamma and IL-5, low IL-2 and no IL-4. As mentioned above, immature DC can induce the development of regulatory T cells [5]. Thus again, pro-inflammatory conditions observed during infection might favor the development of autoimmune disease secondary to the activation of DC, which counteract the development of such regulatory cells.

AICD = activation-induced cell death

Egress of autoaggressive T lymphocyte to peripheral tissue

The presence of autoaggressive effector T cells does not directly imply that tissue damage will ensue. Indeed, effector T cells or pathogenic autoantibodies have still to gain the target tissue. This notion is well illustrated by the Goodpasture syndrome. This syndrome is characterized by the presence of antibodies against the basement membrane and in particular against a non-collagenous domain of type IV collagen molecules. Although this particular molecule is present in the glomerulus, in the lung and in the cochlea, most patients develop nephropathy but only half of them develop pulmonary disorders and none have an auditive defect. This reflects the availability of the target self-antigen, which is readily accessible in the kidney, only accessible in the lung if there is inflammation as observed in patients who smoke [39], and never accessible in the inner ear.

The role of infection in autoimmune disease might also rely on the associated inflammation, which allows extravasation of autoreactive effector cells or pathogenic autoantibodies cells into the target tissue [18]. By an unclear mechanism, autoantibodies can also alter the distribution of T cell-mediated inflammation and lead to destruction of otherwise spared tissue [40].

Concluding remarks

Avoiding tissue damage during autoimmune responses requires a delicate equilibrium between activation and elimination of self-reactive lymphocytes, as well as stringent limitation of the access of lymphocytes to intact tissues. Genetic and environmental factors can probably trigger the development of autoimmune diseases through their effects on some critical tenets for this equilibrium. Certainly, both types of factors influence the clearance of apoptotic cells and the inflammatory reactions that critically determine the availability of self-antigens and their mode of presentation, as well as the extravasation of autoreactive lymphocytes into their target tissues.

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