

Autoimmune odyssey on the Aegean Sea

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The second Aegean conference on 'Autoimmunity: Mechanisms and Novel Treatments' in September, 2005, discussed topics ranging from animal models of autoimmunity to lymphocyte interactions, as well as molecular influences of disease.

The origins of autoimmune disease typically require intracellular interactions not unlike those found in immunity elicited to foreign antigens: a source of self antigen and its presentation, leading to the activation of both B and T lymphocytes^{1,2}. It is now recognized that elements of the innate immune response are also critical for the induction of autoimmunity³. Except for rheumatic fever, the origins and microenvironments that initiate autoantigenic triggers are not understood for either systemic or organ-specific diseases. The key questions addressed at this conference included the following: What forms of self antigen and their presentation initiate autoimmunity? What factors, both above and below the cell surface, trigger autoimmunity versus tolerance or anergy? What are the molecular bases of autoimmunity?

Several investigators presented results on the multifarious nature of B cells in autoimmunity and in their ability to elicit tolerance, to initiate autoreactive responses and to perpetuate the autoimmune response. For example, B cells that present autoantigen are capable of eliciting T cell tolerance, as described by David Scott (Baltimore, USA) in mouse models of diabetes. In technically elegant studies, antigen was engineered into the N terminus of immunoglobulin and was transfected into antigen-

presenting B lymphocytes. Those conditions allow only surface expression of the antigen with very little secreted, leading to induction of T cell tolerance. As in many other studies also presented, the induction of tolerance or autoimmunity in this system is dependent on the presence or absence of conventional B7 costimulation. This approach may also represent a new immunotherapeutic agent for tolerance induction in patients, although it is not yet apparent whether this therapy interferes with epitope spreading or whether tolerance is long lived.

In contrast to tolerance induction, autoantigen-presenting B cells are also capable of activating autoimmune T cells (Alison Finnegan, Chicago, USA; James Thomas, Nashville, Tennessee, USA) or even transferring autoantigen to other 'professional' antigen-presenting cells such as macrophages and dendritic cells (Mark Mamula, New Haven) in propagating the autoimmune response. In the former studies, it was found that proteoglycan directed to the B cell receptor can activate autoimmune T cells and the pathology of arthritis, but that this requires expression of the costimulatory molecules B7-1 and B7-2. In the nonobese diabetic model of diabetes, the ability of insulin-specific B cells to either drive autoimmunity or remain anergic was found to depend on intracellular signals, including the chemokine IP3 and the transcription factor NFAT (Thomas). Mamula presented evidence obtained by two-photon microscopy for the direct transfer of antigen acquired by B cells to dendritic cells and macrophages that in turn amplify the chronic autoimmune T cell response. Overall, these mechanisms provide an explanation for how the B cell receptor, by virtue of its specificity, can focus autoimmunity on selected self proteins and how the ongoing autoimmune



Sunset over the Aegean sea.

response can be maintained with the help of other 'professional' antigen-presenting cells.

Studies regarding the genetic regulation of antigen processing and autoimmunity were presented by Chandra Mohan (Dallas, USA) and Laurence Morel (Gainesville, Florida, USA). Both investigators showed how the field has evolved since early associations of genetic loci were described in models of lupus autoimmunity over the past decade^{4,5}. Three unique loci, *Sle1*, *Sle2* and *Sle3*, have been shown to directly influence B cell and T cell activation, autoantibody production and pathology. T cells from humans with systemic lupus erythematosus (SLE) show a hyperproliferative phenotype manifested by an increased T cell receptor-mediated rise in intracytoplasmic free calcium concentrations and protein tyrosine phosphorylation⁶. In congenic NZM2410 mice, *Sle3* has similarly been associated with increased T cell population expansion and hyperactivity. Mohan presented evidence that *Sle3*-bearing dendritic cells may easily breach tolerance to nuclear antigens and initiate systemic autoimmunity. Of course, the long-term importance of such studies depends on the identification of 'parallel' loci related to the pathology of human disease. In related studies, Chack Yung Yu

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(Columbus, Ohio, USA) showed a correlation between SLE and a low copy number of genes encoding complement 4 protein (C4), with the consequence that patients with SLE have decreased C4 in serum.

Other important macromolecules in the propagation of autoimmunity include the tumor necrosis factor family members B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) found to be increased in human lupus and in the tissue pathology of rheumatoid arthritis. In both spontaneous mouse models and mercury-induced models of lupus autoimmunity, Marc Monestier (Philadelphia, USA) demonstrated increases in BAFF coincident with the production of anti-nucleolar antibodies that could be blocked by TACI-Ig, a BAFF and APRIL antagonist, lending further support to the idea of a key function for B cell development and the use of BAFF-APRIL inhibitors in therapeutic approaches.

Lymphocyte differentiation and signaling

Several sessions were devoted to elucidating how lymphocyte differentiation, homeostasis and signaling are regulated and may be altered in autoimmune disease. Discussions in these sessions included the various factors controlling the fate 'decisions' made by lymphocytes. These topics included the duration of antigen exposure and the cytokine environment and host environment, and how these affect the type of immune response generated through their direct effects on T cell activation.

The ability to generate immunological B cell and T cell memory to self antigen is a central factor involved in the perpetuation of chronic autoimmunity. Studies of developmentally restricted μ MT lpr mice have shown that isotype-switched B cells able to bypass Fas mediated apoptosis can accumulate as an autoimmune memory population (Doron Melamed, Haifa, Israel). Those studies indicate early developmental Fas-induced apoptosis is important in depletion of the autoimmune B cell repertoire.

For T lymphocytes, the nature of the memory response generated can depend on the initial activation and effector response, with the additional complexity that memory T cells are heterogeneous in phenotype, function and tissue distribution. Results presented showed that even brief antigenic exposure is sufficient to generate memory CD4 T cells specific for peptide antigen (Donna Farber, Baltimore, USA) and virus-specific memory CD8 T cells (Matthias von Herrath, La Jolla, California, USA), and both studies showed that the effector-memory subset (often associated with autoimmune diseases) could be generated from these short-term activated T cells. One



Inspirational sunrise over the Aegean sea before the sessions.

difficulty in controlling ongoing autoimmunity is that there are no known strategies that target previously primed effector or memory T cells. The CD28-B7 costimulation pathway, although necessary for initiation of T cell responses and induction of autoimmunity, as discussed above, has been considered dispensable for memory T cell activation. New results were presented demonstrating the susceptibility of memory T cell recall responses to CD28-B7 costimulation blockade by CTLA4-Ig (a fusion protein of the T cell inhibitory protein CTLA4 and immunoglobulin) *in vivo* with predominant effects on the effector-memory subset (Farber), suggesting that the efficacy of this fusion protein in the treatment of rheumatoid arthritis and other autoimmune conditions may be due to direct modulation of autoreactive memory T cells.

Effector responses are influenced by both cellular and molecular factors during antigen encounter. New results were presented showing effects of both interleukin 10 (IL-10) and CD40 on the effector T cell response. Von Herrath showed that blocking responses to IL-10 during viral infection can change a chronic lymphocytic choriomeningitis virus infection into one that is acute and rapidly cleared *in vivo*, suggesting that modulation of this regulatory cytokine early in effector differentiation may have profound effects on the potency of the response. David Wagner (Denver, Colorado, USA) presented results demonstrating that CD40 expression by CD4 T cells marks autoreactive effector cells in diabetes and that

triggering via CD40 on T cells can activate recombination-activating gene expression and cause T cell receptor editing. Another factor regulating effector and memory responses is the host cellular environment, in that lymphopenia can trigger T cell homeostatic proliferation. Charles Surh (San Diego, California, USA) discussed cytokine, costimulatory and host requirements for the homeostasis of naive and regulatory T cells, finding that both populations require IL-7.

Human SLE T cells have many signaling abnormalities that lead to impaired gene transcription. Impaired T cell receptor-mediated signaling in human SLE T cells and normal T cells treated with the SLE-promoting drug hydralazine was shown to be associated with impaired protein kinase C- δ phosphorylation and increased expression of CD70 (Bruce Richardson, Ann Arbor, Michigan, USA). Human SLE T cells have decreased production of IL-2 because *IL2* promoter activity is repressed. New experiments presented at this meeting showed both increased binding of the repressor CREM and decreased binding of the enhancer pCREB to the *IL2* promoter in human SLE T cells. In parallel, increased expression and activity of the phosphatase PP2A dephosphorylates pCREB and limits its binding to the IL-2 promoter. Suppression of the expression of CREM and PP2A by means of small interfering RNA or antisense vectors results in the restoration of IL-2 production (George Tsokos, Silver Spring, Maryland, USA). Increased expression of TRAIL (tumor

necrosis factor–related apoptosis-inducing ligand) occurs in human SLE T cells *in vitro* in the presence of interferon- α , and contributes to abnormally increased apoptotic rates in patients with SLE (Violeta Rus, Baltimore, USA). Yet it is not apparent why activated T cells in humans with SLE do not undergo normal apoptosis but instead resist activation-induced cell death. Insight into that issue may come from the study of pediatric sarcomas. Maria Tsokos (Bethesda, Maryland, USA) showed that sarcoma cells do not respond to TRAIL because of increased survival, which makes them resistant to TRAIL-mediated apoptosis.

C4 deficiency has been long recognized as being among the strongest genetic influences of SLE in humans. Yu showed that humans may have two to ten copies of functional genes encoding C4, that low C4 ‘gene dosage’ leads to decreased C4 protein in the serum and that low C4 ‘gene dosage’ is more prevalent among SLE patients than among people without SLE. A new multiparameter approach for assessing signaling abnormalities in human disease using intracellular staining for a wide panel of signaling intermediates and phosphorylation modifications was presented by Omar Perez (Palo Alto, California, USA). This proteomics approach is intended to identify a phosphorylated-tyrosine signaling ‘signature’ unique not only to subsets of patients with autoimmune disorders but also in cancer and other disease subsets as well.

Interventional therapies and mechanisms

The identification of autoantigens for certain conditions provides therapeutic targets for the modulation of autoreactive lymphocyte function. For example, dimeric major histocompatibility complex class II–peptide chimeras stimulate regulatory T cells to pro-

duce IL-10 and to prevent the development and reverse the progression in diabetic mice (Sofia Casares, New York, USA). In studies using those new reagents, it was found that regulatory T cell numbers decrease with the onset of clinical disease in diabetes, suggesting a model in which transient waves of autoimmune T cell division are linked with the onset of symptoms.

B cell depletion has been introduced in the treatment of SLE and other rheumatic disorders. Data were presented indicating that treatment with an antibody to CD20 preferentially affects the amount of autoantibodies to DNA but not that of other autoantibodies or the titers of protective antibody (Marius Teodorescu, Chicago, Illinois). In addition, the modulation of cytokines can result in substantial changes in the degree and/or expression of autoimmunity. Notably, experimental autoimmune myocarditis is under the control of both T helper type 1 (interferon- γ) and T helper type 2 (IL-13) cytokines, as deficiency of either can promote pathology in the heart (Noel Rose, Baltimore, USA) and in models of arthritis (Henry Hess, Cambridge, Massachusetts, USA).

Finally, statins were reported to alter the T helper type 1–T helper type 2 imbalance in favor of T helper type 2 and to prevent diabetes in a double-transgenic mouse model (Teodor Brumeanu, Bethesda, Maryland, USA). FTY720, a drug that downmodulates G protein–coupled sphingosine–phosphate receptor 1, causing retention of T lymphocytes in lymphoid tissue, was reported to be of clinical value in a phase II clinical trial in patients with multiple sclerosis (Volker Brinkmann, Basel, Switzerland). Those studies suggest that altering T lymphocyte trafficking can likewise have profound effects on suppressing autoimmunity.

Conclusions

The immune system has evolved mainly to protect the host from pathogens. In doing so, a variety of mechanisms have been put in place for immune cells to differentiate what is a foreign and harmful pathogen from the plethora of self proteins and tissues that lymphocytes are required to ‘ignore’. The fact that autoimmunity affects much of the population suggests that the selection mechanisms in place are far from perfect. Perhaps not unexpectedly, this gathering of science further emphasized the need to understand basic mechanisms of disease processes that may later be applied to potential new therapeutic approaches. As with most autoimmune syndromes, the cellular basis of disease is a result of genetic and environmental interactions. Understanding these factors has already shifted the field from broad and nonselective immunosuppression to the targeting of specific cytokines (such as tumor necrosis factor, transforming growth factor- β and BAFF), cell surface costimulatory markers (B7 and inducible costimulator) and lymphocyte cell subsets (B cells and regulatory T cells). As described in the collection of studies presented here, complications in existing or potential new therapies arise in how to control elements of the immune response, such as genetics and immunologic memory to autoantigens, that first evolved to protect the host from pathogens.

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