

melanoma patients vaccinated with peptides of tyrosinase or MelanA. The use of granulocyte-macrophage colony-stimulating factor (GM-CSF) as adjuvant appeared to be critical for the induction of specific HLA-restricted cytotoxic T cells (A. Knuth, Frankfurt). Among the various therapeutic approaches, such as *in vitro* priming of T cells by dendritic cells or gene transfer methods, the German Cancer Aid anti-EpCAM (17-1A) antibody trial (on 185 CRC patients with minimal residual disease) stood out because of its randomized design and seven-year follow-up^{3,4}. The intent-to-treat as well as the eligibility based analysis of this multicentre trial (Dukes' C) showed a gain of 32% in overall survival for the treatment group, and thus not only confirmed but also surpassed the previously reported five-year analysis. Furthermore, this trial lends support to the philosophy

underlying the whole meeting: disseminated tumours are apparently more accessible and less heterogeneous than bulky tumours, and in direct contact with potential effector cells.

Concluding remarks

Although the seminal cells of manifest metastases remain a *terra incognita*, major inroads are being made that undoubtedly will help to avoid the most fatal mistake a strategist can make, namely, to underestimate the enemy.

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Autoantibodies revealed: the role of B cells in autoimmune disease

Freda K. Stevenson and Jacob Natvig



Although autoantibodies are characteristic of many autoimmune diseases, their involvement in the pathological process has long been debated. Recent technology for probing autoantibody structure, together with an increased understanding of the regulation of B-cell activity, have shed new light on the role of B cells in autoimmunity. In addition, the study of ectopic germinal centre sites has revealed disease-associated cellular interactions operating at the local level. Finally, there is immense interest in new clinical approaches to diseases, where treatment has often been limited to relieving symptoms. Therefore, it was important that the meeting brought together scientists and clinicians involved in autoimmunity from basic biology to clinical application.

Genetics and molecular technology have greatly increased our understanding of the cellular processes leading to autoimmunity, and this knowledge is filtering down into clinical applications. At a recent Euroconference, scientists and clinicians focused on the role of B cells in autoimmune disease.*

Normal B-cell behaviour

Normal B-cell differentiation was discussed first to set the scene for understanding the regulatory points that have been evaded in autoimmune disease (T. Rolink, Basel; P. Casali, New York, NY). It is clear that B cells that make autoantibodies, some of which are

directly pathological (D. Isenberg, London; G. Dighiero, Paris; R. Holmdahl, Lund; H. Willison, Glasgow), should have been eliminated or tolerized. Most pathological autoantibodies have undergone isotype switching to IgG. The nature of the IgG subclass will influence the pathological outcome, and is controlled by CD4⁺ T-cell subsets and their cytokines. However, our view of the effects of exogenous cytokines was challenged by I. MacLennan (Birmingham), who presented data indicating local transfer of cytokines between B and T cells.

Activation of 'receptor editing' in systemic lupus erythematosus (SLE)

In patients with SLE, the use of distal V κ genes and downstream J κ genes, together

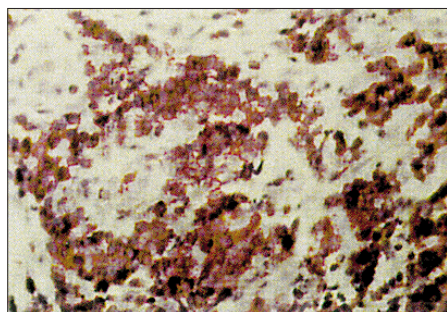


Fig. 1. Histological section of an ectopic germinal centre in synovial tissue of a patient with rheumatoid arthritis. B cells have been stained with anti-CD20. Modified figure reproduced, with permission, from Ref. 3. Magnification = 190 $\times$.

with a low level of somatic mutations in V_{κ} , suggested that B cells were undergoing secondary rearrangements of V_{κ} (P. Lipsky, Dallas, TX). This 'receptor editing' was first described in transgenic mice as a device to delete autoantibody specificity^{1,2}. Further evidence of receptor editing in a wide range of B cells in SLE patients was revealed by the fact that somatic mutations in V_L were considerably less than in the partner V_H (I. Mockridge, Southampton). These unexpected disease-specific findings suggest a hyperactive receptor-editing mechanism in SLE. This raises the question: what is driving this, and how does it relate to induction of autoantibodies?

Autoantibody recognition sites

Immunogenetic approaches have facilitated the probing of autoantibody structures. Phage expression of defined antibodies has allowed manipulation of the amino acid sequence in order to identify those amino acids that are important for recognition. In studies of primary biliary cirrhosis (PBC), the hallmark of which is anti-mitochondrial antibodies, removal of somatic mutations from the antibodies changed the specificity from one site of the target antigen to another (F. Stevenson, Southampton). This suggests that the triggering epitope in PBC differs from the target epitope involved in the developed disease. In mature autoantibodies from SLE or rheumatoid arthritis, the contribution of individual amino acids to antibody recognition, or to expression of idiotopes, can now be mapped (T. Winkler, Erlangen; K. Potter, Southampton; M. Borretzen, Oslo).

This information might have therapeutic relevance.

Activation of immunity

B cells form part of a cellular network, with control of migration mediated by chemokines (L. Silberstein, Philadelphia, PA). Transgenic models are unravelling the way in which the activity and tolerance of B cells can be modulated (D. Eilat, Jerusalem), with a focus on the role of T cells (B. Stockinger, London). The CD40–CD40 ligand (CD40L) interaction is an important influence and, depending on surrounding factors, can lead to stimulation and/or apoptosis (T. Defrance, Lyon). The growing understanding of the mechanisms involved is providing opportunities for therapeutic intervention (T. Kipps, San Diego, CA). CD40 is also expressed by dendritic cells, which are the pivotal cells in 'kick-starting' the immune response and focus on recognition of infectious agents – reminding us that the immune system is mainly concerned with pathogens (P. Ricciardi-Castagnoli, Milan).

Maturation of B cells

The nature and status of the B-cell receptor is of obvious importance in the development of antibody responses, but somatic mutation, which provides increased diversity, could be viewed as a dangerous interference with the genome. The mechanisms of mutation, B-cell development, generation of memory cells and control of aberrant B cells provide the setting from which auto-antibodies can emerge (I. Tomlinson, Cambridge; R. Kuppers, Cologne; M. Neuberger, Cambridge; D. Capra, Oklahoma; H. Schroeder, Birmingham, AL). Signalling through the selected receptor activates complex pathways with molecular connections to the cytoskeleton (J. Wienands, Freiburg). It will be interesting to learn the status of these pathways in autoantibody-producing B cells (F. Caligaris-Cappio, Torino).

Ectopic germinal centres

It is now possible to probe cellular events at the site of disease. This has particular

relevance for autoimmune diseases such as rheumatoid arthritis, where development of ectopic germinal centres in synovial tissue is a feature (Fig. 1) (C. Berek, Berlin). The cytokine milieu in ectopic germinal centres appears unusual, with evidence of a new proinflammatory cytokine, interleukin 17 (IL-17), which induces synoviocytes to produce IL-6 (P. Miossec, Lyon). In addition, chemokines have a role in attracting lymphocytes to the germinal centre site, with dendritic cells being one source (M. Lipp, Berlin; E. Lindhout, Nijmegen). A consequence of these cellular interactions is B-cell activation with clonal expansion, somatic mutation and isotype switch events (J. Natvig and M. Carneiro, Oslo). Similar events occur in salivary glands of patients with Sjogren's syndrome (D. Stott, Glasgow).

Clinical approaches

There are many potential, rational approaches to therapy that are emerging from our increased immunological understanding. However, clinicians currently faced with patients requiring treatment have to be cautious. Monoclonal antibody therapy is now in the clinic, and results from treatment with anti-tumour necrosis factor α (anti-TNF- α) look encouraging for selected autoimmune diseases (R. Foulkes, CellTech, Slough). This approach might also have applications in other situations such as graft rejection. The delivery of new therapies to the clinic can clearly benefit from links with industry and the potential for interaction was a topic of much discussion (R. Foulkes). Another clinical development is to ablate the deleterious immune response in autoimmune patients and treat with bone marrow transplantation (P. Hasler, Basel). This approach is already in clinical trials and the results are awaited with interest.

Conclusion

Our understanding of B cells in autoimmunity has increased in several areas: the route to autoantibody production might begin with crossreacting antigen and pass through a hyperactive receptor-editing checkpoint; B cells have to survive and flourish in a

milieu of surrounding cells and their modulating receptors and factors; specific targeting of these cells for treatment of autoimmune disease will require ingenuity – the European Research Conference has encouraged us to pool our knowledge and resources to move towards this objective.

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The neuroendocrine immune basis of rheumatic diseases

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Systemic rheumatic diseases are characterized by inflammation and microvascular insufficiency and are regulated by complex interactions between different systems, notably the immune, neuronal, endocrine and vascular endothelial systems. The interactions between these systems are widely studied and our understanding of these interactions is improving rapidly^{1,2}.

Hypothalamic–pituitary–adrenal axis

Proinflammatory cytokines such as interleukin 1 (IL-1) and IL-6, as well as eicosanoids such as prostaglandin E₂ and endotoxins, released from inflammatory tissues, activate corticotropin releasing hormone (CRH) at the central, hypothalamic level. This, in turn, stimulates the secretion of circulating corticosteroids (A. Grossman, London). Thus, inflammation stimulates the production of cortisol, which dampens the inflammation (Fig. 1). In rheumatoid arthritis (RA), there is good evidence to suggest that this counter-regulation is relatively ineffective. In this feedback loop two factors play a key regulatory role – CRH and IL-6.

CRH

CRH has not only a central indirect immunosuppressive and an anti-inflammatory effect but also a peripheral proinflammatory effect at local inflammation sites. CRH receptors and mRNA for CRH have been identified in inflammatory cells in the gut and the immune system, and in experimentally-induced in-

There has been much progress in our understanding of the interactions between the nervous, endocrine and immune systems in rheumatic diseases. A recent meeting discussed the advances and their clinical implications in the treatment of these diseases.*

flammation, such as streptococcal cell wall polysaccharide- and adjuvant-induced arthritides (G. Chrousos, Bethesda, MD). In addition, the peripheral nervous system, including postganglionic sympathetic neurons and sensory type C fibres, contributes to local CRH production at inflammatory sites. This finding implicates a link between the stress response and activation of the immune system, as observed in asthma and eczema. At the central level, counter-regulatory pathways modulate CRH activation. Release of CRH is inhibited by both nitric oxide and carbon monoxide, but stimulated by oestrogens. The CRH gene possesses an oestrogen responsive element, and in fact, oestrogens might accentuate CRH stimulation and ultimately increase cortisol production (J. Da Silva, Coimbra).

In addition to CRH, arginine vasopressin (AVP) is secreted by the hypothalamus and helps regulate adrenocorticotropin hormone (ACTH); AVP also has proinflammatory properties. Although AVP secretion is significantly upregulated in RA patients (I. Chikanza, London), it is not yet known whether this up-regulation is primary or a compensatory response to deficient cortisol production³.

IL-6

Cytokines such as IL-6 and IL-1 can increase glucocorticoid secretion by enhancing synthesis and release of CRH and ACTH. However, these cytokines also exert effects at the adrenal level and in human mononuclear and osteoblast-like cells, where they can upregulate the glucocorticoid receptor (A. Angeli, Torino). In active RA, IL-6 levels correlate closely with serum C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and cortisol (G. Neek, Bad Nauheim). Patients with active RA, who were given an oral dose of prednisone (20 mg), exhibited a significant decrease in the levels of IL-6, IL-1 and tumour necrosis factor α (TNF- α) within 4 h of administration of the drug. These reduced cytokine levels were maintained for 24 h and, thereafter, IL-6 levels increased again. Patients with active RA who received

Fig. 1. Simplified scheme of the neuroendocrine–immune interactions in inflamed synovial tissue in rheumatoid arthritis. Stimulation of a pathway is indicated by ‘+’ and inhibition of a pathway is indicated by ‘–’. For example, oestrogens stimulate CRH (thereby stimulating cortisol production), macrophages and Th2 cells, but inhibit Th1 cells, thus shifting the Th1/Th2 balance; all of these activities lead to a slight decrease in inflammation in response to oestrogens. Abbreviations: ACTH, adrenocorticotropin hormone; AVP, arginine vasopressin; calcitonin *grp*, calcitonin gene-related peptide; CRH, corticotropin releasing hormone; IL-1, interleukin 1; Th cells, T helper cells; TNF- α , tumour necrosis factor α .