Meeting Report

Apoptosis and Autoimmunity

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The balance between cell survival and cell death is critical for many aspects of the homeostasis of multicellular organisms. Nowhere is this more so than in the immune system, where the elimination of cells which express self-reactivity is an essential process in the selection of both T cells and B cells during an immune response. Autoimmune diseases are characterised by the production of antibodies against cellular constituents which are not normally recognised as antigens in the majority of healthy people. How may this arise? A number of separable but not unrelated mechanisms may be envisaged (summarized in Figure 1). One is the failure of the immune system to eliminate by apoptosis those lymphocytes which develop reactivity against self-antigens. Another possibility is that novel antigens can appear under certain circumstances, for example following unusual modifications or processing events which affect normal cellular components, and there is a failure of the immune system to recognise these antigens as non-foreign. Apoptosis might be envisaged as a process which could produce such unusual changes in cellular constituents.

These topics, and related issues, were discussed in depth at the seventh Bertine Koperberg Conference held in Nijmegen, The Netherlands during June 17–20, 1999. The meeting covered current views of the mechanisms of apoptosis, the process by which apoptotic cells undergo phagocytosis, the question of links between cell death and autoimmunity, the modifications of autoantigens during apoptosis, and the implications of this growing area of knowledge for the diagnosis and treatment of autoimmune diseases.

A number of presentations addressed the issue of whether defective T cell apoptosis may be a cause of autoimmunity. PH Krammer (Heidelberg) discussed the idea that prolonged T cell activation may make these cells continuously resistant to apoptosis, thus leading to a failure to delete self-reactive T cells. This might be a result of highly elevated levels of IL-2 which can arise in autoimmune disease. This cytokine is known to up-regulate expression of the anti-apoptotic protein bcl-2 in its target cells, leading to inhibition of T cell apoptosis (JS Smolen, Vienna). Another reason why T cells may be less susceptible to apoptotic stimuli may be the presence of mutations in components of the CD95 (Fas/APO-1) system. CD95 is a member of the tumour necrosis factor receptor family that is implicated in the initiation of apoptosis in response to a wide variety of conditions. Defects in the

CD95 pathway are found in autoimmune diseases such as autoimmune lymphoproliferative syndrome (ALPS) (KM Debatin, Ulm). Other components involved in mediating programmed cell death in T cells may also play important roles in the development of autoimmunity. For example, studies with a range of knockout mice have suggested that deficiencies in caspases, the proteases that act as executioners of cell death by cleaving key protein targets, may interfere with apoptosis in T cells (RA Flavell, New Haven). A similar phenomenon is seen in mice that are defective for the JNK protein kinase, a stress-activated enzyme that has been implicated in apoptosis in a number of systems.

An alternative model of how dysregulation of apoptosis may contribute to autoimmunity is that if rates of apoptosis are abnormally high in certain tissues, this may result in the presentation of elevated levels of autoantigens or of new autoantigens to the immune system. Such a hypothesis is not, of course, exclusive of the possibility that T cell apoptosis itself may be impaired, as suggested above. J Savill (Edinburgh) and AA Manfredi (Milan) suggested that the engulfment of apoptotic cells by dendritic cells may result in the presentation of new (auto)antigens to T cells. A further contributing factor could be a failure of phagocytes to clear apoptotic cells expressing multiple autoantigens (for example because of complement deficiency) and this may also result in autoimmunity. The role of phosphatidylserine (PS) as a recognition signal for clearance of apoptotic cells was discussed by V Fadok (Denver). She reported on the cloning of a candidate receptor which could mediate the PS-dependent recognition of apoptotic cells by phagocytes. Defective clearance of apoptotic cells in autoimmune disease, particularly systemic lupus erythematosus (SLE), was discussed by several participants, including M Walport (London), JHM Berden (Nijmegen), E Hack (Amsterdam) and M Herrmann (Erlangen). Rather than exhibiting resistance to apoptosis high numbers of apoptotic T cells and B cells are found in diseases such as SLE (A Perniok, Düsseldorf) and these can trigger the release of inflammatory cytokines such as TNF α , IL-1 β and IL-10 by dendritic cells, as well as causing the maturation of dendritic cells. This combination of events may represent a particularly dangerous response for the development of autoimmunity (Manfredi). However the issue remains controversial since Smolen reported a lack of evidence for increased apoptosis *in vivo* in patients with SLE or scleroderma.

Figure 1 Apoptotic events associated with the development of autoimmunity. The scheme illustrates the variety of factors and processes involving apoptosis that may contribute to the autoimmune response. As discussed in the text, both genetic and environmental influences may cause prolonged T cell activation (associated with reduced apoptosis of these cells). Conversely, other tissues and cell types may exhibit enhanced apoptosis and reduced clearance, leading to the presentation of modified or entirely novel autoantigens. Either process may result in inappropriate and/or enhanced T cell reactivity to cellular epitopes, subsequently leading to an autoimmune response.

If the principal factors that may predispose to autoantibody production are an increased rate of apoptosis combined with a reduced clearance of apoptotic cells, what are the alterations in antigen production that can trigger a crisis leading to disease? KB Elkon (New York) suggested that elevated antigen production may occur following some viral infections, thus providing a basis for the link that has long been suspected between viruses and autoimmunity. Another important question, however, is whether macromolecules that are substrates for degradation during apoptosis can really provide the abnormal antigenic stimuli that can lead to autoimmunity. It is important to note that only a minority of autoantigens are in fact substrates for caspase cleavage during apoptosis (CA Casiano, Loma Linda; A Rosen, Baltimore), and other 'abnormal' products of proteolytic cleavage, such as those produced during necrosis or after exposure of cells to granzyme B, may also be antigenic. Indeed, Rosen showed that granzyme B (released from cytotoxic T lymphocytes) cleaves a much higher fraction of autoantigenic proteins than do the caspases themselves. Many proteins which are caspase substrates (e.g. the DNA-dependent protein

kinase and poly(ADP-ribose) polymerase (PARP)) are also cleaved by granzyme B but the latter enzyme gives rise to different cleavage products, thus generating potentially novel antigenic peptides. S Muller (Strasbourg) mentioned the finding that patient autoantibodies which recognize the Zn-finger region in the N-terminal domain of PARP failed to inhibit the activity of this enzyme. In contrast, they were able to block the cleavage of PARP by caspase-3. A von Mikecz (Düsseldorf) and KM Pollard (La Jolla) discussed aspects of the autoantibody response induced by Hg²⁺ which, in susceptible mice, specifically targets the nucleolar autoantigen fibrillarin. After exposure of cells to HgCl₂, fibrillarin relocates from the nucleolus to the nucleoplasm, where it appears to co-localize with nuclear proteasomes, self-compartmentalizing proteases delivering immunocompetent peptides to the antigen-processing machinery (von Mikecz). This fits well with the observation that during mercury-induced cell death a 19 kDa fragment of fibrillarin is generated. Pollard showed, however, that a tight mercury-fibrillarin interaction is not needed for this cleavage, suggesting that mercury acts on a different level, possibly by activating a specific protease.

Besides the proteolysis mediated by caspases and other enzymes further events can occur during apoptosis which may have a bearing on antigen presentation and the development of autoimmunity. A number of protein kinases are known to be activated as a result of caspase-mediated processing, leading to increased phosphorylation of their protein substrates (as well as perhaps novel substrates). An example of this is seen in the case of the serine/arginine RNA splicing factor complex, components of which exhibit enhanced phosphorylation in apoptotic cells (PJ Utz, Boston). Conversely, the progress of apoptosis can be associated with protein dephosphorylation events that affect known autoantigens such as the La antigen (GJM Pruijn, Nijmegen). Again such changes might be envisaged as giving rise to novel peptides during the degradation of these proteins in certain circumstances. M Piacentini (Rome) discussed the covalent cross-linking of substrate proteins, which in apoptotic cells might stabilize apoptotic bodies, in this way preventing the leakage of their contents.

Apoptosis can also affect the activity of the protein synthetic machinery, perhaps altering the pattern of new gene expression at the translational level (MJ Clemens, London). Similar consequences may result from the specific cleavage of small RNA molecules such as the U1 snRNA (WGJ Degen, Nijmegen) and the cytoplasmic Y RNAs (Prujin). Apoptotic RNA degradation is presumably a result of the caspase-mediated activation of one or more, as yet unknown, specific ribonucleases.

Although we now know a great deal about the mechanisms by which apoptosis is induced, as well as the pathways by which its effects are implemented, it is more difficult to extend this knowledge into clinically relevant aspects of the autoimmune diseases.

D Isenberg (London) reviewed the increasing evidence that phospholipid-protein complexes formed during apoptosis are targeted by pathogenic anti-phospholipid antibodies. The binding and clearance of apoptotic cells by these autoantibodies seems likely to further enhance the anti-phospholipid immune response. Thus abnormalities of apoptosis observed in the course of autoimmune conditions are likely to provide an antigenic stimulus to the production of anti-phospholipid antibodies.

The final section of the conference was able to focus on the use of the induction of apoptosis as a therapeutic principle. In rheumatoid arthritis macrophage-like synovial lining cells appear to play a crucial role in disease expression. Recent studies in experimental arthritis models have shown that these lining cells can be depleted by intra-articular injection of multilamellar liposomes encapsulating the drug dichloromethylene diphosphonate (clodronate) (P van Lent, Nijmegen). The liposomes are phagocytosed by macrophages; thereafter the drug is set free and kills the cells by apoptosis. Induction of experimental arthritis in

lining cell-depleted knee joints was characterized by a significant decrease in the onset of inflammation. Also, during the chronic phase of experimental arthritis, synovial inflammation decreased after injecting the drug-loaded liposomes. A phase I trial in patients with rheumatoid arthritis has shown that depletion of lining cells by clodronate-containing liposomes can be carried out effectively.

Sulphasalazine (SASP) is a drug commonly used in the treatment of inflammatory diseases such as rheumatoid arthritis and Crohn's disease. In both diseases the pro-inflammatory cytokine $\text{TNF}\alpha$, largely produced by macrophages, plays a prominent role. *In vitro* data indicate that SASP can inhibit lipopolysaccharide-induced $\text{TNF}\alpha$ expression in macrophages, both at the mRNA and protein level (RJT Rodenburg, Nijmegen). Intraperitoneal injections of mice with SASP result in the induction of apoptosis of peritoneal (macrophage-like) cells within a few hours. Further studies have indicated that increased caspase-8 activity during SASP-induced apoptosis is involved in the inhibition of $\text{TNF}\alpha$ expression in macrophages. Thus induction of apoptosis of macrophages may contribute to the therapeutic effect of SASP in the treatment of rheumatoid arthritis and other chronic inflammatory diseases. Recent studies in patients with Crohn's disease have indicated that in therapy-resistant cases chimaeric monoclonal anti- $\text{TNF}\alpha$ antibody therapy can induce a clinical response and even lead to complete clinical remission (SJH van Deventer, Amsterdam). This antibody (Infliximab) significantly increases the number of apoptotic mononuclear mucosal cells within 24 h after infusion. These data therefore suggest that one mechanism of action of anti- $\text{TNF}\alpha$ blocking therapies may be the induction of apoptosis of inflammatory cells.

In summary, there is abundant evidence that apoptosis, and related pathways leading to cell death, have important roles to play in the development of autoimmune diseases. The exact mechanisms involved are still open to debate but as the scientific analysis of the apoptotic process progresses it seems likely that new insights will emerge. There is good reason to believe that such developments will in turn lead to the rational application of therapies for a variety of autoimmune and inflammatory conditions.

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