Apoptosis and autoantibodies in systemic lupus erythematosus
Bijl, Marc

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2001

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter XI

Summary and general discussion
Introduction

This thesis studies several aspects of systemic lupus erythematosus dealing with the central question why autoantibodies, responsible for the development of disease manifestations, do occur and what the consequences of their presence are (summarised in chapter I). The development of IgG autoantibodies, as a consequence of a break-through of tolerance, seems to be associated with disturbances in the elimination of cells. Elimination occurs by programmed cell death (apoptosis). Several mouse strains, as the MRL-\textit{lpr/lpr} (lymphoproliferation), and C3H/HeJ-\textit{gld/gld} (generalised lymphoproliferative disease) mice serve as the classical animal models for human SLE. These mice spontaneously develop lymphadenopathy as well as autoimmune features characterised by the presence of autoantibodies to nuclear antigens. It has been recognised that the molecular basis of these abnormalities is a defect in Fas mediated apoptosis. The MRL-\textit{lpr/lpr} mice have a mutation in the Fas molecule that, in the proper genetic background, results in the generation of abnormally spliced mRNA and a severe reduction in functional Fas mRNA. The C3H/HeJ-\textit{gld/gld} mice are deficient in functional Fas ligand (FasL) due to a single base mutation. The defects in Fas and FasL, respectively, result in an incomplete elimination of peripheral autoreactive cells as this elimination occurs predominantly by Fas mediated apoptosis. Initially, based on these animal models, it was supposed that defective apoptosis of, naturally occurring, autoreactive lymphocytes resulted in the development of SLE like features. However, in human SLE mutations in Fas or FasL have been found only incidentally.

Animal studies suggested that, next to the above mentioned mutations, incomplete elimination of (autoreactive) lymphocytes might be due to the presence of soluble Fas (sFas). Soluble Fas is the transcript of Fas mRNA lacking the transmembrane domain because of the deletion of the exon encoding this region. Injecting sFas into female mice of a certain mouse strain resulted in the development of autoimmune features due to blocking of Fas induced apoptosis.

As a first approach to unravel disturbances in the elimination of apoptotic cells in human SLE, in chapter II we evaluated whether levels of sFas are increased prior to a clinical relapse. Assuming that increased levels of sFas, through blocking the Fas-FasL interaction, hinder the induction of Fas-mediated apoptosis in peripheral blood lymphocytes, we hypothesized that in SLE patients prior to a relapse sFas is already elevated. This in turn would than result in the persistence of activated autoreactive lymphocytes followed by an increase in the production of autoantibodies, finally cumulating in a relapse of the disease. Indeed, sFas levels were elevated already 6 months in advance in those patients going to have a relapse. Soluble Fas levels were constantly elevated and might be indicative for the severity of an exacerbation. From this study it could not be determined from which cells sFas originated and whether there is a relation of sFas levels with activation markers on immune cells.
Therefore, in chapter III we evaluated the relation between sFas levels, disease activity, and lymphocyte activation markers. We demonstrated that levels of sFas in patients with SLE are increased, even during quiescent disease. Levels of sFas correlated with disease activity scores and with the extent of activation of circulating B cells. These findings suggest that sFas levels are a reflection of cell activation. Since elevated sFas levels have been found in a considerable number of other, non-autoimmune diseases it can be concluded that elevation of sFas is not specific for SLE or rheumatic diseases in general. Furthermore, although sFas levels in SLE patients were elevated, the amounts of circulating sFas were fairly low. Taken together, these data render a pathophysiological role for sFas in SLE doubtful.

Blockade of Fas-mediated apoptosis induction by sFas in SLE does not seem to be a very relevant factor in the etiopathogenesis of the disease. As a consequence it can be speculated that in SLE a defective elimination of autoreactive lymphocytes is not contributing to or even causing the disease. Nevertheless disturbances in apoptosis might have pathophysiological consequences. Especially the finding that on apoptotic cells autoantigens, whether or not modified, are presented had great impact on our understanding of the disease. It can be hypothesized that the persistence of apoptotic cells, either by increased production or by decreased clearance results in continuous presentation of autoantigens so breaking tolerance. In line with this hypothesis it might be assumed that in SLE increased apoptosis occurs because factors promoting apoptosis-induction are present. This theory was supported by studies reporting increased proportions of apoptotic lymphocytes and neutrophils in the peripheral blood of SLE patients. To analyse the susceptibility of lymphocytes for apoptosis induction mediated by Fas, we analysed membrane Fas expression in SLE patients and controls (chapter IV). We showed that in SLE patients the percentage of peripheral blood B-lymphocytes (CD19+) expressing Fas was increased and was related to the state of lymphocyte activation and the extent of disease activity. In addition, the results are compatible with the concept that upregulation of membrane Fas renders B-lymphocytes more susceptible for apoptosis resulting in increased rates of apoptotic peripheral blood lymphocytes as described in SLE.

During the studies dealing with Fas expression on peripheral blood lymphocytes it was noticed that Fas expression of smoking controls, especially on B cells, was higher compared to that in non-smokers. Differences in Fas expression, as stated, might influence susceptibility for Fas-mediated apoptosis. As alterations in the humoral immune response and increased susceptibility for infections have been demonstrated in smokers, we wondered whether smoking changed Fas expression and, subsequently, levels of apoptosis after Fas-induced apoptosis. Indeed, the initial observation of increased Fas expression on peripheral blood B-lymphocytes in smoking individuals could be reproduced in 10, otherwise healthy, smokers (chapter V). Furthermore, we demonstrated that smoking was
also associated with elevated percentages of Fas expressing CD4⁺ T-lymphocytes. In addition, we showed that, using agonistic anti-Fas monoclonal antibodies, the Fas pathway in smokers is functionally intact. These findings might suggest that activated lymphocytes will be eliminated at a higher rate in smokers. The increase in Fas expression on these lymphocyte subsets therefore might have consequences for the immune response of smoking individuals. Furthermore, these findings can be of relevance interpreting data of Fas expression and Fas-induced apoptosis of lymphocytes in various populations of patients.

So far, we investigated whether sFas levels, smoking and activity of SLE could be of influence in the induction of apoptosis by ligation of the Fas receptor. In chapter VI we analysed whether there are intrinsic abnormalities in activation-induced and Fas-induced apoptosis in SLE patients. To avoid the influence of disease activity only SLE patients with inactive disease were included for this study. As expected, stimulation with anti-CD3 resulted in up-regulation of membrane Fas in patients and in controls. After proper up-regulation of Fas, cells were incubated with anti-Fas. In vitro induction of apoptosis by anti-CD3 as well as by anti-Fas occurred both in SLE patients and controls, and was higher in SLE patients after incubation with both anti-CD3 as well as with anti-Fas. Fas expression and in vitro induction of apoptosis therefore are increased in SLE even in the absence of disease activity. As in SLE patients, even during inactive disease, elevated proportions of activated peripheral blood lymphocytes can be demonstrated it seems prudent to conclude that these results reflect increased in vivo activation of peripheral blood lymphocytes and that no intrinsic defect in the apoptotic machinery of SLE patients is present.

Antibody production in SLE is antigen-driven and T cell dependent. Activation of T cells requires interaction of the T cell receptor (TCR) with the major histocompability complex (MHC) molecule complexed with the antigenic peptides in the presence of additional signalling through costimulatory molecules. Of these, the CD28-CD80/CD86 and CD40-CD40L pathways are well known. The relevance of costimulation in the development of autoimmune disease in animal models is supported by several studies in which costimulation antagonists have been used as an effective approach to treat these diseases. We hypothesized that, in SLE patients, an increase in the expression of costimulatory molecules facilitates T- and B-cell activation and survival, increases autoantibody production, and may so influence the development of autoimmune disease. In line with this hypothesis we measured the expression of costimulatory molecules on peripheral blood lymphocytes in lupus patients and healthy controls in relation to the state of lymphocyte activation to address the following questions: is there an increase in expression of costimulatory molecules in patients compared to controls and are these changes related to the state of lymphocyte activation, disease activity and levels of antibodies to dsDNA. Results are given in chapter VII. In SLE patients, the expression of
CD86 on CD19+ B cells was increased and was associated with disease activity, B cell activation and levels of anti-dsDNA. We suppose that the increased CD86 expression will render (autoreactive) B cells more susceptible for T cell-help. This might prolong survival of activated B cells and will facilitate autoantibody production resulting in increased amounts of autoantibodies produced.

Of the autoantibodies produced, those directed to nucleohistone and their components (e.g. dsDNA) are strongly implicated in the pathogenesis of SLE. For example, these antibodies can be eluted from kidney specimens of lupus mice with overt glomerulonephritis. IgG class antibodies seem most relevant as there is a close relation between levels of IgG anti-dsDNA and histologic activity scores in patients with lupus nephritis. Furthermore, in the majority of patients, a renal relapse is preceded by a significant rise of IgG anti-dsDNA.

All IgG subclasses can be found in kidney biopsies. Of the different subclasses, IgG1 and IgG3 activate complement more efficiently than IgG2 while IgG4 does not activate complement at all. The IgG subclass distribution of autoantibodies could therefore be of relevance in the pathogenesis of lupus nephritis. In chapter VIII we describe the evaluation of their possible nephritogenic role. We monitored levels of total IgG and IgG subclasses of antibodies to nucleohistone and to dsDNA in time in patients who suffered a renal relapse. IgG subclass distribution in kidney biopsies of patients with a renal relapse was assessed as well. Serological data obtained from patients with renal relapses were compared with those of lupus patients who developed an extra-renal relapse, in order to evaluate the specificity of the findings for lupus nephritis.

The important role that IgG2 autoantibodies to nucleohistone might play in the initiation and outcome of lupus nephritis leads to the question whether the handling of the specific IgG subclasses differs between SLE patients and controls and between the individual SLE patients. Handling of IgG is accomplished by Fcγ receptors (FcγR). Several polymorphisms of these receptors have been recognised, each with its own functional consequences. In particular the polymorphism of the second Fcγ-receptor (FcγRIIa), caused by the presence of either arginin or histidin at position 131 (FcγRIIa-R131 and FcγRII-H131, respectively) determines the interaction with IgG2 and, to a lesser extent, IgG3. In contrast to FcγRIIa-H131, the FcγRIIa-R131 isoform can not interact with IgG2 and has less affinity for IgG3. The FcγRIIIa-receptor has a valine (V) to phenylalanine (F) change at amino acid position 158. Individuals homozygous VV for FcγRIIIa bind more IgG1 and IgG3 compared with those homozygous FF for FcγRIIIa. Finally, the four amino
acid changes termed neutrophil antigen polymorphism (NA1 and NA2) for FcγRIIIb have consequences in the handling of IgG subclasses as individuals homozygous NA1 phagocytose IgG1 and IgG3 opsonized particles more efficiently than individuals homozygous NA2 for FcγRIIIb. To evaluate associations between FcγR polymorphisms and disease susceptibility we determined FcγR polymorphisms in a strictly Caucasian SLE population and matched controls from the same region. Furthermore, we analysed whether the respective FcγR polymorphisms were associated with specific disease manifestations and whether these polymorphisms determined the clearance of immune complexes in vivo. In chapter IX we show that of the known FcγR polymorphisms only the R-H polymorphism of FcγRIIa is a relatively minor determinant of susceptibility to SLE and affects the clearance of immune complexes in vivo. The V-F polymorphism of FcγRIIIa influences the development of a set of clinical disease manifestations including arthritis, serositis and hematological abnormalities.

In summary, no defects in the initiation of apoptosis or in the apoptotic machinery could be found in human SLE. In contrast, susceptibility of peripheral blood lymphocytes for (anti-CD3- and anti-Fas-induced) apoptosis in SLE seem to be increased, probably due to their increased in vivo activation. Next to the increased production of apoptotic cells a decreased clearance of these cells might be present in SLE. This combination can result in the accumulation of apoptotic cells, subsequently resulting in the formation of autoantibodies directed to the presented (nuclear) antigens. Among these, the antibodies to nucleohistones and dsDNA are most important. Their production might be facilitated through increased expression of CD86 on the cell membrane of B cells in SLE patients. Of the autoantibodies produced the IgG1- and IgG2 subclasses are probably most relevant. Their presence might have implications for the course of the disease as the handling of the IgG subclasses is dependent on genetically determined Fcγ receptor polymorphism. In chapter X the evolving concepts about the pathogenesis of SLE are reviewed.

Crucial in research dealing with SLE are two questions. First, what induces autoimmunity, that is what genetical factors predispose an individual to develop SLE and which environmental factors are, in addition, necessary to break tolerance. Secondly, which factors determine the very heterogeneous disease manifestations.

Concerning the first question many studies are nowadays being performed in which genome scans of lupus patients are made to analyse which genetical factors might predispose an individual for autoimmune diseases. Progress has been made and several regions have been identified which contribute to disease susceptibility. Among these are the HLA loci, the complement genes, and, possibly, Fcγ-receptors. It is still speculative, but there are compelling data that the gene products of these loci might have a function in (disturbed) elimination of apoptotic cells by phagocytosis and presentation of autoantigens. At present, we are performing studies in SLE patients in which in vivo as
well as in vitro phagocytosis of apoptotic cells is analysed. The data from genome scans
gathered so far support the hypothesis that multiple genes are involved and that the
genetics of SLE resembles that of many other complex genetic diseases. Fine mapping
and candidate gene sequencing efforts in the chromosomal intervals identified are at
present performed in our own laboratory of medical genetics. Hopefully these studies will
lead to the identification of major genes and their respective products, predisposing to
human SLE. Furthermore, knowledge about these genetic factors makes it possible to
analyse much more specifically which environmental factors play an additional role in the
development of SLE. Among these, sunlight exposure and the use of certain drugs are
commonly accepted.

It is important to disclose whether prior to or at the moment SLE gets clinically manifest,
alterations in the immune system are present and can be demonstrated. This touches the
important dilemma how to interpret the immunological disturbances found in SLE
patients. Are they cause of the disease or caused by the disease? This is not easily
resolved. However, whenever it is possible to identify individuals at risk to develop SLE
analysis of alterations in the immune system already present before disease expression can
be evaluated. Many items dealt with in this thesis hopefully can be answered whenever
studies in persons susceptible for autoimmune disease can be performed. That is, are
increased levels of apoptotic cells present in the peripheral blood or in the tissues already
prior to the formation of autoantibodies? Are signs of lymphocyte activation and
disturbances in the expression of cell membrane molecules such as Fas, CD80 and CD86
already present in these individuals?

This brings up the second question: which factors determine disease manifestations? It is
suspected that also genetical factors might influence the course of the disease as we have
shown for the polymorphism of the Fcγ-RIIIa receptor. Probably, patients at risk to
develop severe organ involvement can be identified which gives the opportunity to treat
immunological disturbances in advance. Recently, the introduction of biologicals has been
a great therapeutical step forward in the treatment of many disorders, including
autoimmune diseases. Intervention with monoclonal antibodies will allow us in the near
future to intervene specifically, and to correct immunological disturbances also in patients
suffering from SLE.

In conclusion, increasing knowledge about the course of SLE in combination with the
availability of therapeutics which make intervention in the immune system much more
specific will profoundly change follow-up and treatment of patients with SLE. In the near
future major progress in our understanding of the pathogenesis of SLE is expected. This
will result not only in changes in treatment and follow-up of those patients already known
with SLE but will also have its impact on their family members. It is supposed that early
detection of family members at risk for developing autoimmune diseases will give us the
opportunity to intervene at an early phase thereby preventing (overt) disease.