Anti-neutrophil cytoplasmic antibodies in idiopathic inflammatory disorders
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This thesis studies the prevalence of anti-neutrophil cytoplasmic antibodies (ANCA) in idiopathic inflammatory disorders in which GS-ANA were described during the 60s: rheumatoid arthritis, autoimmune liver diseases, inflammatory bowel disease, and juvenile chronic arthritis. In all of these disorders the presence of ANCA was related to disease activity, disease duration and severity of disease. Furthermore, the antigenic specificity of ANCA in chronic inflammatory disorders was studied. In addition, this thesis investigated the pathophysiological significance of ANCA in chronic inflammatory disorders, by studying the capacity of ANCA to activate granulocytes.

Chapter 1 is a review in which target antigens of ANCA and the clinical significance of the different ANCA specificities are discussed. The classical or cytoplasmic ANCA (c-ANCA) is in almost all of the cases directed against proteinase 3. C-ANCA occur in more than 90% of patients with extended Wegener's granulomatosis (WG), in 75% of patients with limited WG, and in some 40 to 50% of patients with vasculitic overlap syndromes. The presence of c-ANCA is highly specific for those diseases and changes in levels of c-ANCA precede disease activity. Perinuclear ANCA (p-ANCA) are directed against different cytoplasmic constituents of neutrophils and occur in a wide range of diseases. p-ANCA directed against myeloperoxidase have a high specificity for necrotizing vasculitides, but may incidentally occur in other diseases as well. p-ANCA directed against elastase are incidentally found in patients with vasculitic disorders, whereas lactoferrin antibodies are detected in patients with primary sclerosing cholangitis with or without ulcerative colitis, and in rheumatoid arthritis. p-ANCA of undefined specificity can be detected in ulcerative colitis, Crohn's disease, autoimmune liver diseases, chronic arthritides and in some 5% of healthy controls. The diagnostic value of p-ANCA of undefined specificity has to await characterization of the antigens involved.

Chapter 2 studies the prevalence, interrelationships and target antigens of ANCA in rheumatoid arthritis, and relates the presence of ANCA to disease duration and occurrence of extra-articular manifestations. ANCA were detected in 70% of rheumatoid sera, whereas in 36% of the sera the antibodies reacted with cytoplasmic constituents of the neutrophil as shown by a cytoplasmic staining pattern on paraformaldehyde fixed granulocytes. Elisa studies showed that 20% of the sera reacted with lactoferrin, 1% with myeloperoxidase, and 1% with elastase. Western blotting studies confirmed the Elisa data and revealed reactivity with hitherto unknown polypeptides of 67/66 kD (6%) and 63/54 kD (9%). Neither of the antibodies was associated with a particular subset of disease, but the prevalence of the antibodies tended to increase with longstanding disease. Anti-lactoferrin antibodies were demonstrated to be present in synovial fluid as well.

Chapter 3.1 studies the diagnostic significance of ANCA in chronic liver diseases by testing sera from patients with primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), autoimmune chronic hepatitis (AI-CAH) and non-autoimmune liver diseases. ANCA were detected in 28% of PBC sera, in 79% of PSC sera, and in 88% of AI-CAH sera, while they were not detected in non-autoimmune liver disease sera. The presence of ANCA was significantly correlated with the presence of cirrhosis. The ANCA antigens involved were not Pr3, MPO or HLE, but Western blotting studies showed reactivity with lactoferrin, the 67/66 kD doublet and a 40 kD protein. Reactivity with either of these proteins was observed in sera from patients with PBC (20%), PSC (38%) and AI-CAH (17%).

Chapter 3.2 approaches the possible immunopathogenetic importance of ANCA in PSC by studying the occurrence of ANCA in patients with PSC after liver transplantation. Contamination and that the ANCA can be directed against lactoferrin and that the antibodies are reactive with lactoferrin. No relation was observed between the development of cirrhosis and the presence of ANCA.

Chapter 4.1 studies the prevalence of ANCA in juvenile inflammatory tissue diseases. Primed PR3, MPO and HLE were not detected in any of the control sera. Onset of disease activity was frequently determined by elevations of cytoplasmic fluorescence and nuclear fluorescence of patients with PBC, MPO or HLE were demonstrated in sera from patients with lactoferrin.

Chapter 5 describes the diagnostic importance of ANCA in autoimmune liver diseases. Primed PR3, MPO and HLE were not detected in sera from patients with autoimmune liver diseases. Not all ANCA...
ANCA in the 60s: the occurrence of juvenile disease specificity of ANCA (c-ANCA) in more patients than in sera from controls. Levels of c-ANCA against lactoferrin without triggering vasculitis are reported in sera from patients with inflammatory disorders. The antigenic specificity of ANCA was investigated by Elisa, showing lactoferrin or myeloperoxidase as activating antigens. So, within the spectrum of IBD, the presence of p-ANCA is not specific for UC. When titers of ANCA are taken into account, the presence of high-titered p-ANCA suggests active UC.

Chapter 4.2 studies the antigenic specificity of ANCA in IBD. 76% of the p-ANCA positive sera in UC and 50% of the p-ANCA positive sera in CD showed cytoplasmic fluorescence on paraformaldehyde fixed neutrophils, indicating that indeed cytoplasmic antigens are recognized by a considerable number of these sera. Western blot analysis showed reactivity with either lactoferrin, the 67/66 kD doublet or the 63/54 kD doublet in 46% of the UC sera and in 32% of the CD sera. Since identical patterns of reactivity have been observed in rheumatoid arthritis sera and autoimmune liver disease sera these data suggest that ANCA of restricted specificities are not specific for IBD but are present in diverse conditions characterized by chronic inflammation.

Chapter 5 describes the prevalence of ANCA in juvenile chronic arthritis (JCA) and other juvenile inflammatory disorders such as cystic fibrosis, juvenile diabetes and connective tissue diseases. p-ANCA are detected in 35% of JCA sera and in only 6% of the disease control sera. Considering the onset type of JCA, ANCA are detected in 44% of oligoarticular onset JCA, in 36% of polyarticular onset JCA, and in 16% of systemic onset JCA. No relation was observed between ANCA and either the presence of rheumatoid factor, prolonged disease or more progressive disease. However, ANCA were significantly less frequently detected during remission of JCA suggesting that ANCA might be a marker of disease activity. On paraformaldehyde fixed neutrophils only 14% of the JCA sera showed cytoplasmic fluorescence, while 23% of the sera showed nuclear fluorescence. The nuclear fluorescence pattern was related with the presence of ANA, especially in the sera of patients with polyarticular onset JCA. Antigen specificity studies showed that PR3, MPO or HLE are not the antigens involved. Western blotting studies showed reactivity with lactoferrin (5%) or polypeptides of 67/66 kD (9%).

Chapter 6.1 studies neutrophil activation by ANCA, and analyzes the underlying mechanism. Primed normal donor neutrophils are demonstrated to express the ANCA antigens PR3, MPO and lactoferrin on the cell surface, and interaction of these antigens with their respective antibodies resulted in activation of the neutrophils as demonstrated by the production of superoxide. In contrast to other reports, activation of neutrophils by ANCA depended on the presence of the Fc-part of the antibodies, and the availability of the Fcy-receptors on the neutrophils. Especially the second Fcy-receptor appeared to be involved. Not all ANCA positive samples were capable of granulocyte activation irrespective of the
ANCA titer. Interestingly, samples capable of granulocyte activation contained relatively high levels of the IgG3 subclass of ANCA in contrary to the ANCA positive samples that could not induce activation.

Chapter 6.2 extends the studies of chapter 6.1. Paired ANCA positive samples from active and inactive disease were studied for their capacity to induce the respiratory burst, and granulocyte activation was related to ANCA titer and subclass distribution, together with disease activity. Sera of patients with active disease better induced the respiratory burst in primed neutrophils than sera of patients with inactive disease. There was a significant relation between ANCA titer and the amount of superoxide produced. In addition, changes in IgG3-subclass of ANCA appeared to correlate with changes in superoxide production. Therefore, not only changes in ANCA titer during the course of disease, but also changes in levels of IgG3-subclass of ANCA are important as the latter are directly related to the neutrophil activating capacity of the autoantibodies.

The final chapter of this thesis, chapter 7, is an overall discussion on the data generated in this thesis. It concludes with the vicious circle of ANCA associated idiopathic inflammatory disorders: ANCA are capable of activation of primed neutrophils. This results in respiratory burst and degranulation. The ANCA antigens are now available for immune complex formation and new antibodies can be produced.

With respect to the questions posed in chapter 1.2 we conclude:
1. ANCA in the idiopathic inflammatory disorders have comparable antigenic specificities, in particular lactoferrin and polypeptide doublets of 67/66 and 63/54 kD, suggesting that these ANCA are not specific for one disease entity. ANCA of those specificities rather seem a marker for inflammatory disorders on an autoimmune background.
2. ANCA in rheumatoid arthritis and autoimmune liver diseases are related to either disease duration or severity of disease. This relation is less clear for ANCA in the inflammatory bowel diseases and juvenile chronic active hepatitis.
3. The pathogenetic role of ANCA remains in part to be elucidated. Nevertheless, ANCA can activate primed neutrophils. This process is Fc-dependent and particularly exerted by the IgG3 subclass of ANCA. These data suggest that ANCA are capable of maintaining or even amplifying the inflammatory process.