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New developments in the pathogenesis of systemic vasculitis

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The vasculitides include a highly heterogeneous group of clinicopathological entities. Ranging from benign, self-limiting disorders to life-threatening conditions, the vasculitides share a common histopathologic substrate; the inflammation of blood vessels, which may involve vessels of any size throughout the vascular system.

Largely unknown etiological agents among which viruses, drugs and other environmental agents can be considered, trigger a cascade of immunopathogenical mechanisms able to damage the vessel wall. In addition to classically recognized immune complex deposition and complement activation, these include the generation of antineutrophil cytoplasmic antibodies, antiendothelial cell antibodies and a T-cell-mediated immune response directed against putative antigens potentially present in the vessel wall. These and other potential immunopathogenic mechanisms are not mutually exclusive and they probably act in concert to sustain and reinforce vessel damage triggered by still elusive and probably heterogeneous agents.

Etiolopical agents

It is well known that lesions caused by fungal and bacterial infections *(i.e.,* aspergillus, tuberculosis) may include vasculitic phenomena. An association between hepatitis B virus infection and the development of classical polyarteritis nodosa has been observed for many years. Recently, a strong association between hepatitis C virus infection and mixed cryoglobulinemia has been recognized. Other viral infections that have been related to the occurrence of vasculitis are HIV, cytomegalovirus and parvovirus B19. Identifying viruses as potential etiological agents in vasculitis may have important therapeutic implications.

Immunonathogenic mechanisms

Immune comolex deposition

Many years ago, experimental animal models, such as the Arthus phenomenon and serum sickness, provided interesting insights into the potential of immune complexes to damage the vessel wall. According to this model, immune complexes would activate the complement cascade with the generation of chemotactic products. These would attract neutrophils which, in turn, would damage the vessel wall by releasing lysosomic enzymes and reactive oxygen species.

Antineutroohil cytoolasmic antibodies

One of the most intriguing discoveries in the field of vasculitis has been the recognition of an association between Wegener's granulomatosis and microscopic polyangiltis and the presence of circulating antineutrophil cytoplasmic antibodies (ANCA). Accumulated experience has revealed that ANCA potentiate neutrophil-mediated vessel damage both *in vitro* and in animal models. ANCA-mediated immunopathogenic mechanisms will be discussed in depth in the next lecture.

Antiendothelial cell antibodies

Antiendothelial cell antibodies (AECA) have been described in a variety of systemic vasculitis, as well as in autoimmune diseases with vascular involvement. AECA exhibit different functional activities on endothelial cells. Some are able to induce an activated phenotype in cultured endothelial cells while others can induce complement-activated endothelial cell lysis. Antigens recognized by AECA seem to be highly heterogeneous and await further characterization.

T-cell-mediated immune response

Immunopathogenic studies have shown that, in several vasculitis syndromes, inflammatory infiltrates are mainly composed by activated T lymphocytes and macrophages. Even in the processes where neutrophils are thought to have a prominent role, such as immune complex-mediated vasculitis or ANCA-associated vasculitis, at some point, inflammatory infiltrates disclose a seemingly high amount of mononuclear cells. The mechanisms through which T lymphocytes and macrophages are activated to produce vessel injury are heterogeneous. In temporal arteritis lesions, the identification of clonal expansion of a minority of infiltrating T lymphocytes suggests a specific immune response directed against antigens present in the vessel wall. In Takayasu arteritis, heat shock protein recognition by 7/6 T lymphocytes may contribute to vessel damage.

Vascular response to inflammation

Vessel wall components, particularly endothelial cells, actively and dynamically react to the products released by infiltrating leukocytes. During the past few years, it has become apparent that endothelial cell response to cytokines and growth factors amplifies the inflammatory response by three main mechanisms: expression of adhesion molecules for leukocytes, additional cytokine and

growth factor production and angiogenesis. Inflammation-induced neovessels are prominent in vasculitis and are the main site of adhesion molecule expression. Therefore, newly formed vessels contribute to the development of vascular inflammatory infiltrates by recruiting leukocytes. On the other hand, angiogenesis may prevent isohemia by providing new blood supply.

Vascular response to inflammation may eventually lead to vessel occlusion generating ischemia in tissues supplied by involved vessels. Organ schemia is the main cause of organ dysfunction and serious complications in patients with vasculitis. Vessel occlusion may develop through spasm, thrombosis and, more frequently, intimal hyperplasia and fibrosis. Several cytokines and growth tactor with prothrombotic, vasoactive and tibrogenic properties have been demonstrated to be produced in vasculitis lesions.

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Antineutrophil cytoplasmic autoantibodies in pathology: Major autoantigens and disease associations

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Antineutrophil cytoplasmic autoantibodies (ANCA) were described in 1982 by Davies and coworkers in patients with necrotizing glomerulonephritis (GN). In 1985, Van der Woude and coworkers reported ANCA as being a characteristic marker of Wegeners granulomatosis. In the 1990s, ANCA have become a commonly used diagnostic test and the term ANCA-associated vasculitides, including Wegeners granulomatosis, microscopic polyanglitis and Churg-Strauss syndrome, as well as pauci-immune necrotizing crescentic GN, has been generally accepted. In addition to being an important diagnostic tool, it has been shown that the dynamic of ANCA titers in the majority of patients reflects the activity of the disease. In view of the clinical serologic correlation, as well as recent *in vitro* and *in vivo* experimental studies, ANCA presumably play a key role in the pathogenesis of and vascular lesions in ANCA-associated diseases. However, positive ANCA occasionally occur, most probably as an epiphenomenon, in a variety of pathological conditions, such as inflammatory bowel diseases, autoimmune liver diseases, connective tissue diseases, tumors, infectious diseases and others.

Detection of ANCA

The standard approach for the detection of ANCA is the indirect immunofluorescence (IIE) technique followed by one of the antigen-specific quantitative assays.

IF is performed on ethanol-fixed normal human leukocytes. Routinely, immunoglobulin (Ig)G antineutrophil antibodies are detected, although in some pathologic conditions, 1gM or IgA isotypes should also be tested. Two main patterns are observed by IIE: cytoplasmic (C-ANCA) and perinuclear (P-ANCA). P-ANCA, which is actually an artifact of alcohol fixation, can be confused with positive antinuclear antibodies, so a control testing on Hep-2 cells is needed. Atypical C- and atypical P-ANCA patterns have also been described.

ANCA antigen specificity is quantitatively determined by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay or Western blotting technique.

ANCA antigens

It has been established that ANCA are directed against various proteins, mostly enzymes in the cytoplasm of neutrophils and monocytes. The most common, and diagnostically the most important, are myeloperoxidase (MPO) and serin protease proteinase 3 (PR3). Both are contained in azurophilic granules and are translocated to the cell surface during the activation of neutrophils. Anti-MPO antibodies mostly exhibit P-ANCA pattern (128/171 in our study), rarely atypical F- (12/171) or atypical C- (26/171) and exceptionally C-ANCA (1 /1 71). Anti-PR3 antibodies regularly show C- (46/69) or atypical C-ANCA (14/69) and rarely P- or atypical F-ANCA patterns in IIE.

Other known ANCA antigens, such as lactoferrin, elastase, lysozyme, cathepsin G, rs-enolase, azurocidin, bactericidal permeability increasing protein, human lysosomal-associated membrane protein 2 (h-lamp-2) and defensin occur rarely, according to the literature in less than 5%. In addition, there is still a conspicuous number of ANCA positive sera with undetermined antigen specificity.

The majority of ANCA-positive sera are specific for a single antigen, although the co-occurrence of different ANCA antigens has not been studied systematically. In our large series of 374 ANCA-positive patients, 31 exhibited simultaneous positivity for anti-MPO and anti-PR3, lithe association of anti-MPO or anti-PR3 antibodies with rare specificities and four the co-occurrence of rare ANCA antigen specificities. In addition, the specificity of ANCA changed in the course of the dis6ase in five patients.

ANCA in classification of vasculitides

It has long been recognized that pathological lesions in diseases that are now determined as ANCA-associated share many light and immunohistological similarities. Vascular lesions in various