From immunohistochemistry to pathophysiology of rheumatoid arthritis: Cross reactivity of self anti-immunoglobulin antibodies with collagen(s) may contribute to mechanisms of connective tissue damage (a hypothesis)

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Abstract

The mechanism of initiation and development of rheumatoid arthritis was a subject of several hypotheses. None of those hypotheses has, however, convincingly explained all important facts, related to clinical, immunological and pathological aspects of the disease. A hypothesis is presented here, suggesting that, in the course of rheumatoid arthritis, an immune system produces anti-immunoglobulin antibodies, cross-reacting with self collagen(s). This cross-reactivity may be a significant part of the complex set of pathological phenomena, characteristic for rheumatoid arthritis. The hypothesis originated from the author's own observations of binding of the anti-immunoglobulin antibodies to the fibrous connective tissue (presumably to collagens contained in it) in histological sections, subjected to immunohistochemical procedures.

Key words: rheumatoid arthritis, immunoglobulins, collagen, connective tissue, histology.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of joints with an involvement of the heart, the lungs, the brain, the eyes, nerves and bone marrow [1, 2]. A large body of knowledge about RA has been acquired during the last century as a result of clinical, pathologic, immunologic and genetic studies of RA patients [1, 2, 3]. It has been complemented with studies of experimentally-induced arthritis in mice [4, 5, 6]. Today we know that the susceptibility to RA depends on the allelic variability of genes, coding for MHC class II molecules [3], on genes, coding for a number of cytokines [2, 5], and on other factors, like age and gender [1, 2]. The aetiology of RA is not yet, however, known. Among the unsolved problems is a question what the primary "arthritogenic molecule" is which initiates a cascade of events, resulting ultimately in destruction of joints [1, 2, 3, 4, 6]. The candidate molecules include: collagen(s), certain epitopes of MHC class II molecules (QKRAA motif or "the shared epitope" [3]), the component(s) of staphylococcal cell wall, the Epstein-Barr virus protein, and other [1, 2]. A comprehensive pathophysiological theory of RA has to be built upon these facts and hypotheses; they may be imagined as elements of a jigsaw puzzle, with some pieces of bizarre shape, and some other missing. That is why new observations, experiments and theoretical concepts are desired.

Hypothesis: The hypothesis, as presented here, has originated from a reflection on the peculiar immunohistochemical artefact, observed by the author and his associates [7, 8], namely, the binding of the anti-immunoglobulin antibodies (anti-Ig Abs) to a fibrous connective tissue, presumably to collagen(s) of this tissue (Figs 1, 2, 3). One may ask if a similar phenomenon occurs in vivo and, if so, whether it plays some role in the pathophysiological mechanisms of RA.

Hypothesis: In the course of rheumatoid arthritis (RA), the immune system produces anti-immunoglobulin antibodies (anti-Ig Abs) which cross-react with self collagen(s); this cross reactivity contributes to a complex set of pathologic phenomena, constituting RA, and thus, it may be a factor involved in causing damage of the connective tissues in joints.

The hypothesis is provisional but I believe it has an operational utility - it may help raise a number of questions both for theoretical consideration and practical testing. (1) Can a collagen-binding antibody be induced not only by a foreign collagen but by another, non-collagen foreign antigen? (2) May a collagen be auto-immunogenic and capable of inducing anti-collagen

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Figure 1. Binding of an anti-immunoglobulin antibody to endomysium. The unfixed cryostat section of a quadriceps muscle of the mdx mouse [9] was incubated with the HRP-conjugated goat antibody against mouse immunoglobulins (DACO, code No. P 447) [7]. The brown precipitate (a product of reaction catalyzed by HRP) surrounds individual muscle fibers, so its localization corresponds to endomysium (see Fig. 2 for comparison). Magnification 600x. Comments: The reaction is interpreted as due to the cross-reactivity of anti-Ig antibodies with collagen(s) [7, 8].

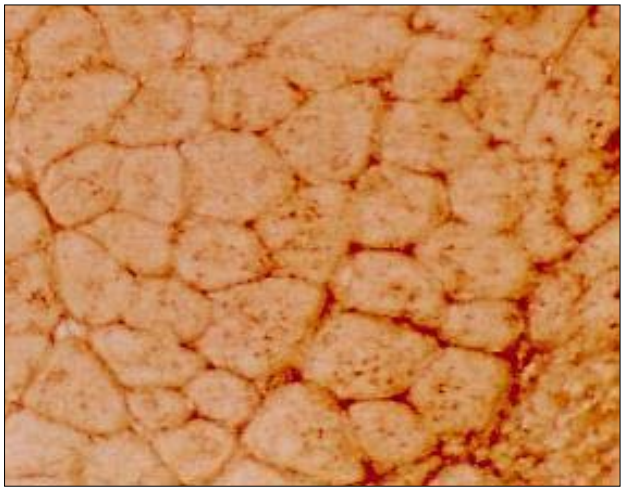


Figure 2. Localization of collagen in skeletal muscle. The quadriceps muscle of the mdx mouse [9] was fixed in Bouin's fluid, embedded in paraffin, cut into sections, deparaffinized and stained with Sirius red [7, 10]. Magnification 600x. Comments: The staining is specific for collagen [10], so, red color indicates localization of collagen(s) (in endomysium), similarly to the localization of sites, binding the anti-Ig antibodies, as seen in Fig. 1.

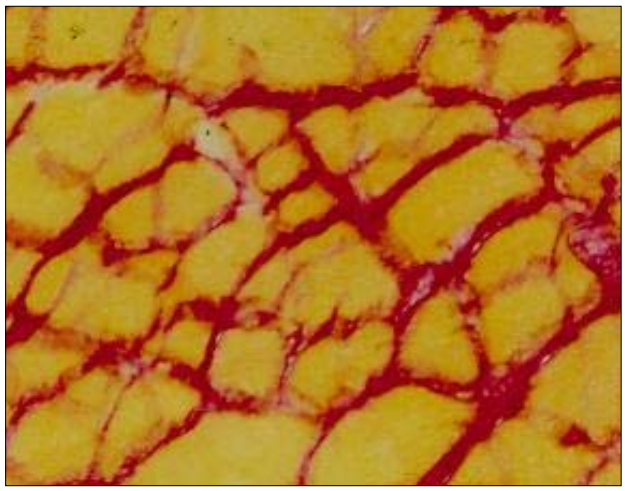
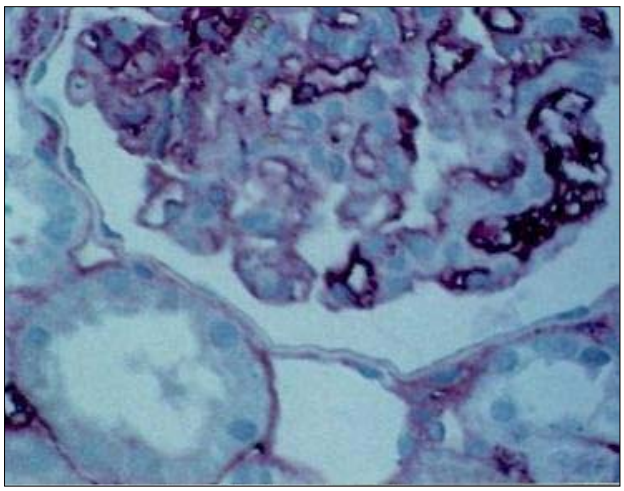


Figure 3. Immunohistochemical artifact in the test for IgG deposits in human kidney. Histological section of a formalin-fixed, paraffin-embedded sample of kidney (kindly provided by dr. Anna Sulikowska-Rowińska, University Medical School, Warsaw). Magnification 450x. Steps of immunostaining [11]: (a) incubation with the primary, unlabelled, rabbit/mouse antibodies specific for human IgG; (b) incubation with the secondary, biotinylated, goat antibody against rabbit/mouse Ig; (c) incubation with Streptavidine, conjugated with alkaline phosphatase; (d) visualization of alkaline phosphatase activity with Fast Red system (all reagents from DAKO). Cell nuclei and cytoplasm stained blue with haematoxylin. The reddish-brown reaction product, deposited over connective tissue, surrounding renal tubules, and over walls of capillary vessels (presumably over basement membranes) in a glomerulus. Comments: In the light of other tests performed on the same biopsy, the reaction seen in Fig. 3 is an artifact. Localization of IgG in the stroma of kidney and in the walls of glomerular capillaries was not confirmed by other tests. Instead, a presence of immune complexes in mesangium has been revealed Dr Sulikowska-Rowińska, personal communication. The explanation of the artifact: the primary or the secondary antibody or both cross-reacted with collagens of the stromal connective tissue and of the capillary basement membrane.



antibodies, which cross-react with immunoglobulins? (3) Do susceptibility to RA depend, in addition to the genes, already known as the ones, controlling this susceptibility, also on genes, coding for collagens and/or for factors, controlling post-translational modifications of potentially immunogenic motifs on collagen molecules? (4) Can the anti-Ig Abs, while cross-reacting with collagen, interfere with a normal structure of the network of extracellular fibers in connective tissues? (5) Do the anti-idiotypic antibodies against the anti-collagen antibodies play any role in RA induction or development? (Note that antibodies of this type should bind, theoretically, certain collagen ligands, since the secondary anti-idiotypic antibodies may mimic epitopes of the original immunogene).

The above questions were intended to inspire scientists with new concepts, relevant to pathophysiology of RA, a disease, upsetting the life of many patients and disturbing the minds of all rheumatologists.

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