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Prevalence of HLA-DR3 and Autoantibodies to Connective Tissue Components in Dupuytren's Contracture[1]

JOSEF NEUMÜLLER,* JOHANNES MENZEL,† AND HANNO MILLESI‡

*Ludwig Boltzmann Institute of Rheumatology and Balneology, P.O. Box 78, A-1107 Vienna-Oberlaa, Austria; †Institute of Immunology, University of Vienna, A-1090 Vienna, Austria; and ‡Ludwig Boltzmann Institute of Experimental Plastic Surgery, 1st Surgical Department, University Hospital Vienna, A-1090 Vienna, Austria

Forty-six patients with Dupuytren's contracture (DC) and 55 control persons were HLA-typed for class I and HLA-DR class II antigens and were investigated for the presence of autoantibodies against elastin (ELAB) and collagen types I–IV (ACA I–IV). Using the χ^2 test, obtained from 2 × 2 contingency tables, a significant association was found between DC and HLA-DR3 and autoantibodies to types I–IV collagen. ELAB and ACA I and III were significantly correlated with HLA-DR3 in the whole group of patients plus controls. In analogy to other diseases with autoimmunologic features the presence of HLA-DR3 therefore seems to indicate a higher risk for the formation of connective tissue autoantibodies. The remodeling processes during the course of fibrosis in DC might be responsible for this autoantibody formation.

INTRODUCTION

Dupuytren's contracture (DC) is a disorder of the palmar aponeurosis. It belongs to the complex of fibromatous and fibrotic diseases. Although it is well characterized from the pathological point of view, its ethiopathogenesis is unknown.

The most striking alterations in this disease are by contracted longitudinal bands in the palmar aponeurosis (1–5). These bands are contracted and interrupted by nodules containing myofibroblasts with a typical spacial arrangement. These cells show a characteristic phenotype similar to muscle cells and are thought to play a role in the contracture of the palmar aponeurosis (6). Furthermore, not only cells but also structural elements of the extracellular matrix, like collagen and elastic fibers, are altered in DC (7). Thus, the palmar fascia of DC patients contains increased amounts of type III collagen (8–10). Although usually no significant infiltration of the connective tissue by inflammatory cells occurs, Józsa *et al.* (11) described mononuclear cells, containing IgG and IgA, around the DC nodules and IgM containing cells perivascularly. Furthermore, granulocytes in the surroundings of the DC lesions were found by Bartal *et al.* (12).

The suggestion that Dupuytren's contracture might be associated with an autoimmune response to collagens was made by Gay and Gay (13). Assays for serum antibodies to collagen confirmed that some of the patients indeed had these antibodies (14–15).

Genetic studies in families of patients suffering from DC revealed a familiar increase in the incidence of this disease (16–18). In such a situation it is useful to search for a genetic marker which is polymorphic and associated with the disease. One of the most polymorphic human genetic systems is the human leucocyte antigens (HLA) complex. A few years after the detection of this system associations with several rheumatic, dermatologic, gastrointestinal, and neurologic diseases with certain HLA-antigens were observed (for an overview see 19–20). A remarkable number of these disorders belongs to the autoimmune diseases where cellular and humoral immune reactions are directed against the autologous tissue. Such reactions may play either a central or merely a marginal role in the pathogenesis of the respective diseases. As far as an association between HLA and DC is concerned, no strong and distinct association with any HLA antigen has so far been found (15, 21–24).

In contrast to rheumatoid arthritis, which is also characterized by autoantibodies against connective tissue components (25–27) in DC, only a few publications exist about as association between HLA and those autoantibodies. The aim of our study was therefore to investigate whether statistically significant associations can be demonstrated between HLA antigens of the classes I and II, DC, and autoantibodies to antigens of the connective tissue.

MATERIALS AND METHODS

Patients

Forty-six patients [40 males and 6 females, aged between 31 and 77 years (60.1 ± 10.6)] suffering from DC

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who underwent a surgical therapy and 55 healthy volunteers [37 males and 18 females, aged between 29 and 81 years (50.1 ± 13.4)] were selected for this study. HLA typing was performed in 33 DC patients and 38 control persons. Autoantibodies against elastin (ELAB) were determined in 40 DC patients and 53 control persons. Autoantibodies against collagen (ACA) I II were determined in 22 DC patients and 43 control persons. Both HLA typing and determination of ELAB were carried out in 28 DC patients and 36 control persons. Both HLA typing and determination of ACA I–IV were carried out in 15 DC patients and 33 control persons.

Determination of ELAB by Enzyme Immunoassay (EIA)

Solubilized forms of elastin retain their immunological reactivity and can therefore be used for the detection of ELAB in solid-phase assays. Among the several published methods to solubilize elastin we chose the procedure described by Robert and Poullain (28). Starting material was a commercial preparation of insoluble elastin from bovine ligamentum nuchae (Sigma E 1625, St. Louis, MO). Soluble kappa 2 elastin was prepared as follows: elastin powder (10 g) was suspended in 250 ml of 1 *N* KOH/ethanol (80:20, v/v) and stirred at 37°C for 80 min. After centrifugation at 16,000 *g* for 15 min the pellet was resuspended in KOH/ethanol and again heated for 60 min. The neutralized supernatant was lyophilized and redissolved in 0.02 *M* acetic acid. The soluble elastin was chromatographed on a Sephacryl S 200 column (Pharmacia, Uppsala, Sweden) of the dimensions 1.8 × 26.0 cm. Calibration was performed with human IgG and bovine serum albumin as molecular weight markers. For the assay of ELAB the molecular weight fraction between 40,000 and 65,000 (corresponding to the maximal molecular weight eluted) was used as a soluble elastin antigen in the EIA procedure. To this end, the wells of microtiter plates (Nunc, Roskilde, Denmark) were coated with 0.2 ml of the kappa 2 elastin solution of defined molecular weight range (0.1 mg/ml in pH 7.5 phosphate buffer or Tris buffer, 0.02 *M*, containing 0.45 *M* NaCl and 0.02% sodium azide). Incubation was for 3 days at 4°C. The coated plates were washed and incubated for 2 hr at room temperature with phosphate-buffered saline (PBS), containing 0.4% bovine serum albumin, to reduce nonspecific binding effects. After washing, 0.2 ml of a 1:20 dilution with PBS/0.1% Tween 20 of each sample to be tested was added to each well (triplicate assays). Incubation was for 30 min at 37°C and 60 min at 4°C. After threefold washing the second antibody, a 1:1000 dilution of antihuman IgG, coupled to horseradish peroxidase, in PBS–BSA (1%) without azide was added. Incubation and washing cycles were as above. Finally, the substrate 2,2′azinobis-(3-ethylbenzthiazoline sulfonic acid) was added and extinction measured in an ELISA reader (Multiscan LCC/340, Flow, Helsinki, Finland) at 405 nm after 10–30 min. Each result is reported as the mean OD of triplicate assays. The limit for positivity was defined as mean OD of the controls plus the threefold standard deviation.

Determination of ACA by EIA

ACA to collagen types I–IV were determined by a procedure analogous to that used for ELAB. Coating was done at a much lower antigen concentration (5 μg/ml). Collagen type I was prepared from human infant dura mater, type II from human cartilage, type III from human skin, and type IV was a commercial preparation from human placenta (Sigma C 7521). All collagens were extracted by peptic digestion and purified by salt fractionation (29). They were used only in native form as EIA antigens.

HLA Typing

The determination of HLA antigens of the class I (HLA-A, -B, and -C locus) was performed using the microlymphocytotoxicity test (MLCT), NIH standard technique (30), while testing of the class II HLA antigens (only HLA-DR subregion) was performed by the double-fluorescence MLCT (31). As recommended, in most cases every specificity was tested using three antisera of different serum charges. Only antisera which were obtained commercially and which had been tested by the producer were used for this investigation. Specificities are listed in Table 1.

TABLE 1

Specificities of Antibodies Used for HLA Typing

HLA class I
HLA A locus
A1, A2, A2 + A28, A3, A9, A23(9), A10, A25(10), A25(10) + A66(10), A26(10), A11, A29(19), A29(19) + A1, A30(19) + A31(19)
HLA B locus
Bw4, Bw6, B5, B51(5), B5 + B35, B5 + B49(21), B5 + B18 + B35, B7, B7 + B22, B7 + B55(22), B8, B12, B44(12), B45(12), B13, B14, B15, B38(16) + A9, B39(16), B16 + A10, B17, B17 + B63(15), B57(17), B18, B21, B21 + B52(5), B49(21), B22, B55(22), B56(22), B27, B35, B37, B39(16), B40, B40 + B13, B60(40), B44(12), B55(22), B56(22)
HLA C locus
Cw2, Cw3, Cw4, Cw5
HLA class II
HLA DR subregion
DR1, DR2, DR3, DR4, DR5, DR7, DR52, DR53
Control sera
HLA class I positive, HLA class I negative, HLA class II positive

Statistics

As is usual in HLA investigations, the χ^2 test obtained from 2 × 2 contingency tables was carried out (32). If some fields of the contingency tables were occupied by numbers <10 a correction according to Yates (33) was performed.

From the 2 × 2 contingency tables a relative risk was calculated as follows:

(The concordance of disease and antigen × number of times there is no disease and no antigen)/(discordance of disease and antigen × number of times there is antigen but no disease)(19).

RESULTS

The incidence of autoantibodies to elastin and to the collagen types I–IV in DC patients and in control patients is shown in Table 2 and Fig. 1. The measurement values in terms of OD are illustrated in Fig. 2.

Among the group of 33 DC patients and 38 control persons typed for HLA antigens of class I and II, only the HLA-DR3 was associated with DC ($P < 0.05$) giving a RR of 2.94.

The statistical evaluation of the concordance between DC and autoantibodies against antigens of the connective tissue by the χ^2 test revealed the association summarized in Table 2.

From the statistical evaluation we cannot conclude whether the HLA-DR3 or the autoantibodies against elastin and collagen, on the one hand, or the disease, on the other hand, are responsible for an enhanced RR. Nevertheless, ACA I were significantly and ACA II, III, and IV highly significantly associated with DC yielding enhanced RR values. When comparing the autoantibodies against collagen or elastin with the HLA-DR3 in DC patients only, no statistically significant association could be demonstrated. Although HLA-DR3 and autoantibodies against collagen and elastin also occurred in apparently healthy persons, they were increased in DC (Fig. 1). Interestingly, HLA-DR3 and ACA I as well as ACA III were significantly associated ($P < 0.05$ and $P < 0.01$, respectively) in the control group of the healthy persons. In the statistical analysis comprising all the individuals tested (DC patients + controls) significant associations between HLA-DR3 and ELAB ($P < 0.05$) as well as between HLA-DR3 and ACA I ($P < 0.01$) could be demonstrated. Also, although HLA class I-antigens are in linkage disequilibrium with HLA-DR3 [in Caucasians HLA-A1, -B8, -Cw7 (34–35)], no other HLA antigen was found to be associated with DC or with any autoantibody we have investigated in this study.

In follow-up studies involving three patients we observed that ELAB (Fig. 3) and ACA III (Fig. 4) completely disappeared 9 months after the operation.

TABLE 2

2 × 2 Contingency Tables

(a) Comparison of the presence of HLA-DR3 in DC patients and controls

	DC	C	Σ	
HLA-DR3$^+$	19	12	31	$\chi^2 = 3.85$
HLA-DR3$^-$	14	26	40	$P < 0.05$
Σ	33	38	71	RR = 2.94

(b) Comparison of the presence of ELAB and ACA I–IV in DC patients and controls

	DC	C	Σ	
ELAB$^+$	14	7	21	χ^2 (Y)[a] = 5.04
ELAB$^-$	26	46	72	$P < 0.05$
Σ	40	53	93	RR = 3.54
ACA I$^+$	9	6	15	χ^2 (Y) = 4.54
ACA I$^-$	13	37	50	$P < 0.05$
Σ	22	43	65	RR = 4.27
ACA II$^+$	6	1	7	χ^2 (Y) = 7.01
ACA II$^-$	16	42	58	$P < 0.01$
Σ	22	43	65	RR = 15.75
ACA III$^+$	10	4	14	χ^2 (Y) = 9.21
ACA III$^-$	12	39	51	$P < 0.01$
Σ	22	43	65	RR = 8.13
ACA IV$^+$	7	1	8	χ^2 (Y) = 9.16
ACA IV$^-$	15	42	57	$P < 0.01$
Σ	22	43	65	RR = 19.6

(c) Comparison of the presence of ELAB and ACA I–IV in both groups, DC patients and controls together

	HLA-DR3$^+$	HLA-DR3$^-$	Σ	
ELAB$^+$	11	4	15	χ^2 (Y)[a] = 2.16
ELAB$^-$	19	30	49	$P < 0.05$
Σ	30	34	64	
ACA I$^+$	12	1	13	χ^2 (Y) = 18.74
ACA I$^-$	8	27	35	$P < 0.01$
Σ	20	28	48	
ACA II$^+$	3	1	4	$\chi^2 = 0.78$
ACA II$^-$	17	27	44	not significant
Σ	20	28	48	
ACA III$^+$	8	3	11	χ^2 (Y) = 4.13
ACA III$^-$	12	25	37	$P < 0.05$
Σ	20	28	48	
ACA IV$^+$	4	2	6	$\chi^2 = 0.94$
ACA IV$^-$	16	26	42	not significant
Σ	20	28	48	

[a] The χ^2 values were corrected according to Yates.

DISCUSSION

It has been known for some time that DC may be familial (16–18). An early study (21) showing no association with the HLA system was followed by two investigations including the HLA-DR subregion (22, 24). According to these publications, there is a raised overall preference of HLA-DR3. We observed a statistically significant association only with HLA-DR3 ($P < 0.05$), resulting in a relative risk of 2.94 for persons with this HLA antigen to incur the disease.

There is a striking association of HLA class II genes with autoimmune diseases. This implies that HLA genes themselves are among the predisposing agents or that, alternatively, HLA variants may simply be genetic markers in linkage disequilibrium with other putative susceptibility alleles (36–38).

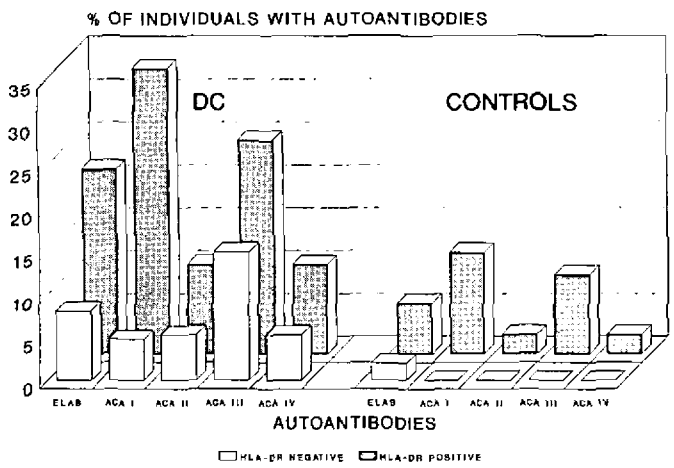

FIG. 1. ELAB and ACA I–IV and positivity for HLA-DR3 in DC patients and controls. The heights of bars indicate the percentage of HLA-DR3$^+$ (shadowed bars) and HLA-DR3$^-$ (empty bars) Dp patients or control persons.

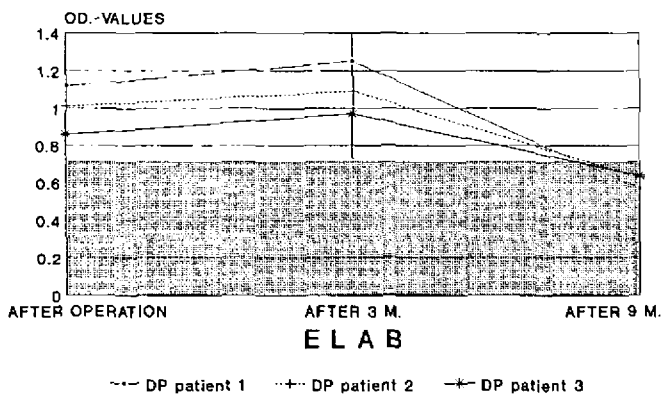

FIG. 3. Time course of autoantibodies to elastin in three DC patients. The measurement values are indicated in terms of OD. The shadowed area indicates the normal range (the upper limit being defined as mean OD values in control persons plus 3 SD).

Some controversy exists in the literature about the significance of cellular immune reactions in DC. In this respect the question arises whether the tissues involved contain a sufficient number of inflammatory cells to mount a local immune response against the connective tissue components undergoing an intense remodeling process. According to Józsa *et al.* (11), Andrew *et al.* (39) and Baird *et al.* (40), both lymphocytes (T- and B-cells) and macrophages are present in and/or around the nodules. According to Baird *et al.* (41), a significant percentage of these cells expresses HLA-DR molecules, which implies the potential ability of macrophages to present antigen to T-cells and to release fibroblast-stimulating cytokines. Hence, the local cell population in or around the nodules could be able to mount an autoimmune response directed against soluble antigens (denatured collagen or elastin peptides).

In 1991, Andrew *et al.* (39) found lymphocytes only in the tissue around nodules, but found monocytes inside of the nodules. However, one should consider that the stage of the disease strongly influences the vascularization and the remodeling process in the affected tissue. The demonstration of cells from collagenase-digested tissues of the subcutaneous tissue of DC patients (41) does not give any clues to the origin of the cells (inside the vessels or in the surrounding connective tissue). However, in our own investigations (7) using light and electron microscopy, we found infiltrating inflammatory cells only rarely in sections of fascial strands or nodules of the palmar aponeurosis. The amount of mononuclear cells present in the extravasal tissue of the palmar aponeurosis of DC patients is much lower than that found in other autoimmune diseases such as the inflamed synovial membrane of patients suffering from rheumatoid arthritis. It has therefore been pointed out by Andrew *et al.* (39), who obtained similar results, that in respect to the small number of macrophages in DC tissues "it seems un-

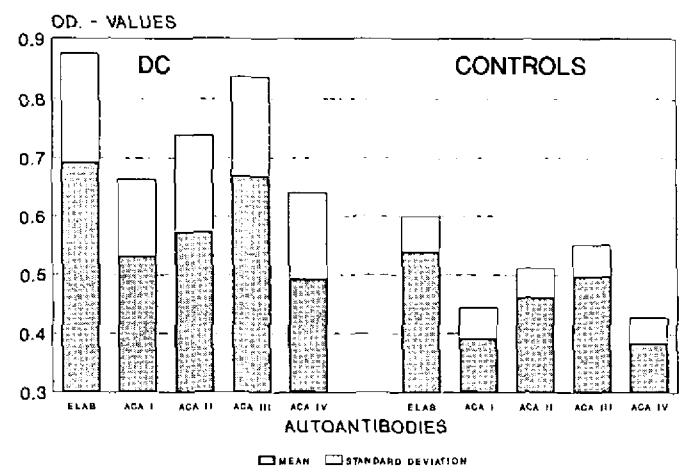

FIG. 2. Autoantibodies to elastin and collagen types I–IV. The measurement values are indicated in terms of OD.

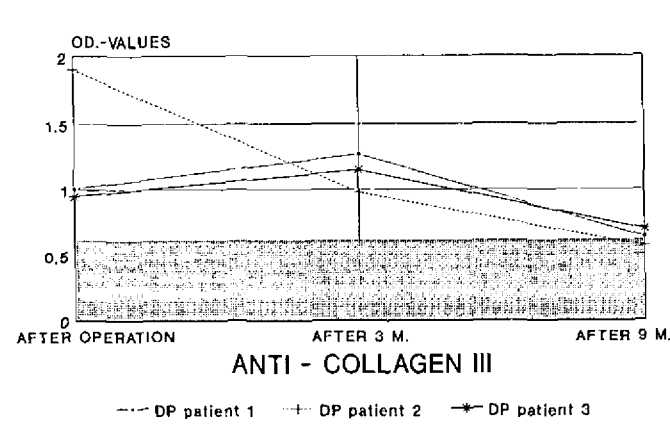

FIG. 4. Time course of collagen III autoantibodies in three DC patients. The measurement values are indicated in terms of OD. The shadowed area indicates the normal range (the upper limit being defined as mean OD values in control persons plus 3 SD).

likely that macrophage derived growth factors are the sole stimulus for the proliferation of fibroblasts in this disease."

Interestingly, we (7) observed that the repair process of the palmar aponeurosis of DC patients is characterized by the occurrence of an increased number of mast cells, above all in the subcutaneous tissue closely beneath the strands and nodules affected by the disease. Mast cells are activated by acute trauma (42) which is discussed to be a precipitating event initiating the DC. This initiation results in the secretion of vasoactive substances, cytokines, and growth factors such as IL-1, IL-3, TNF-α, TGF-β, and GM-CSF (43). Mast cells are found in an increased frequency in most of the fibrotic disorders (44, 45). Their mere presence at sites of increased tissue turnover does not imply that they are playing an active role in the pathogenesis of these processes. However, a partial mast cell degranulation as it has been found in some of the DC tissue specimens investigated by us (7) suggests a mast cell activation. The release of mast cell secretagogues, e.g., of histamine, is able to stimulate the growth of fibroblasts (46), to enhance collagen synthesis *in vitro* (47) and *in vivo* (48), and to initiate angiogenesis (49–51) which is a striking feature in fibromatous areas of the aponeurosis of DC patients. Among other cytokines and growth factors, TGF-β, as the most fibrogenic cytokine, is secreted by monocytes/macrophages as well as by mast cells (43, 52, 53) and could play an essential role in the pathogenesis of DC. Recently, Baird *et al.* (40) demonstrated an increased production of IL-1α and -1β, TGF-β, and b-FGF in DC biopsies, all of them cytokines which are able to stimulate fibroblast growth and to modulate the formation of components of the extracellular matrix. TNF-α and IL-1 can also induce the expression of selectins and integrins on the surface of monocytes (54) and endothelial cells (55). Even though an enhanced expression of adhesion molecules has not yet been demonstrated in tissues affected by DC, an alteration of the microvasculature, such as a bulging of the endothelial cells into the lumen, has been described (39, 56, 57).

Although DC cannot be regarded as one of the classical autoimmune diseases, autoimmune phenomena, such as ELAB and collagen with different type-related specificity (ACA), could be demonstrated. However, follow-up studies on patients involved in this study showed that these autoantibodies disappeared several months after surgical intervention, indicating their transitory nature. This observation supports the hypothesis that the removal of the diseased tissue, and therefore, the source of antigens from denaturated collagen, by surgical treatment reduces the stimulus for the production of autoantibodies against collagen. As demonstrated in our follow-up studies there is, however, a transitory peak of autoantibody production which might be the consequence of surgical intervention. Nevertheless, the mere demonstration of autoantibodies to connective tissue components does not necessarily imply that these autoantibodies are instrumental in the pathogenesis of the disease.

The basis for collagen autoimmunity seems to be an immunogenetic predisposition. Thus, it was shown by Klimiuk *et al.* (25) and Sanders *et al.* (58) that rheumatoid arthritis (RA) patients presenting with autoantibodies to native type II collagen represent a distinct genetic subset, characterized by an association with HLA-DR3. In contrast, antibodies to denatured type II collagen are associated with HLA-DR4, the class II HLA antigen to which susceptibility for RA has been strongly linked (26). An association between the presence of autoantibodies against denatured type II collagen and HLA-DR4 was demonstrated by Pereira *et al.* (15) in patients with DC. In contrast to this investigation, we detected ACA to type I and type III collagen in higher frequency than ACA to type II collagen. This seems logical, since both these interstitial collagens are subject to an intense remodeling process in the course of DC, including neosynthesis and degradation by collagenase and other tissue proteases. Type II collagen, on the other hand, is absent in the normal palmar fascia and its appearance in the affected aponeurosis is a rare finding (5). As far as associations to the HLA system are concerned, an association between HLA-DR3 and ACA to type I and III collagens in the control group and also in the whole group of DC patients plus controls has been found by us. Due to the low number of DC patients who were both HLA typed and tested for ACA no statistically significant associations were seen in the group of DC patients alone.

ELAB were not significantly associated with HLA-DR3 in either DC patients or controls. In the whole group, however, a statistically significant association was found. Antibodies to kappa elastin of bovine origin were first described in sera of severely atheromatous patients by Stein *et al.* (59). They were also detected in patients with thromboangiitis obliterans, another disease showing significant association with the HLA system (60).

Therefore, the higher frequency of ELAB and ACA in HLA-DR3$^+$ DC patients strongly supports the hypothesis of an immunogenetic background of DC. Thus, we suggest, in conclusion, that immune cells in cooperation with mast cells could play an essential role in the pathogenesis of DC. A genetic predisposition, characterized by the presence of HLA-DR3, might favor the occurrence of autoimmune phenomena such as the formation of autoantibodies against components of the extracellular matrix. These autoantibodies could contribute to the activation of immune cells.

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