

Granulocyte-Specific Antinuclear Antibodies

Possible Significance for the Pathogenesis, Clinical Features and Diagnosis of Rheumatoid Arthritis

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1. INTRODUCTION

The main manifestation of rheumatoid arthritis (RA) is chronic progressive articular inflammation clinically characterized by joint stiffness, pain, swelling and local hyperthermia, and pathologically by joint effusion, synovial membrane hypertrophy and articular cartilage destruction (81, 246). In addition a number of extraarticular tissues and organs may be affected by rheumatoid inflammatory

processes rendering the disease pattern a true systemic one involving skin, mucous and serous membranes, various parts of the locomotor apparatus including nerves, muscles and bones, the lymphoid and haematopoietic systems and a variety of internal organs (27, 75, 86).

Observations made in the last two decades have indicated involvement of immune mechanisms in the pathogenesis of RA (14, 43, 77, 90, 236, 239, 246). Polymorphonuclear and mononuclear phagocytes are thought to contribute substantially to such immunologically mediated inflammation (reviewed in 3, 88, 212, 214). The present communication intends to focus on a facet of phagocytic cell

Abbreviations: ANA, antinuclear antibodies; CF, complement fixing; DNA, deoxyribonucleic acid; DNA-ase, deoxyribonuclease; GS-, granulocyte-specific; Ig, immunoglobulin; ON-, organ-nonspecific; PBS, phosphate buffered saline; PBSMg, phosphate buffered saline with MgCl₂; RNA, ribonucleic acid; RNA-ase, ribonuclease.

pathology only sporadically dealt with in the past by which phagocytic cells—possibly in a posthumous state—may contribute to inflammation by appearing as antigens giving rise to autoantibody production. The review will cover most of the available data on antinuclear antibodies specifically reacting with nuclei of mature phagocytes of the peripheral blood, the so-called granulocyte-specific antinuclear antibodies (GS-ANA). The main emphasis is put on the immunoglobulin (Ig) class and subclass diversity, the complement fixing (CF) properties of GS-ANA and their participation in immune complexes in an attempt to conceptualize their possible role in the immunopathogenesis of RA and for special clinical features such as neutropenia in RA. The survey will also deal with methodology used for their demonstration and characterize GS-ANA occurring in health and disease.

2. DEFINITION, TERMINOLOGY AND REACTIVITY OF GS-ANA

GS-ANA are antibodies capable of reacting *in vitro* with nuclei of mature neutrophilic granulocytes, sometimes also with monocytes and/or eosinophilic granulocytes, but not with lymphocytes or with tissue cells of human or animal origin (161, 224, 227). Such antibodies are most often found in sera of patients with RA (see section 8). The reactivity is generally restricted to the mature cells mentioned; however, some sera of patients with Felty's syndrome, i.e. RA, leucopenia and splenomegaly (61), have exhibited reactivity even with metamyelocytes and myelocytes of normal bone marrow and peripheral blood of chronic myeloid leukaemia patients (50, 57, 222). In these sera weak reactions have been shown with monocytes of Schilling's type monocyte leukaemia, but not with myeloblasts of acute myeloblastic leukaemia (222). In the following, ANA reacting with nuclei of lymphocytes and/or human thyroid or rat liver will be collectively termed organ-nonspecific (ON-) ANA. Granulocyte-reactive ANA is a term used by some authors to indicate ANA

reacting with granulocyte nuclei in the presence or absence of ON-ANA (13, 16, 51, 53, 93, 97, 130, 202, 211).

Sera are considered GS-ANA positive if all reactivity is directed to phagocyte nuclei as stated above or if this specific reactivity supercedes reactivity with other cell nuclei by at least two dilution steps (164, 173, 224). Svec (184) defined GS-ANA somewhat differently, restricting the term to ANA retaining the above reactivity after absorption with nucleoprotein.

A precise biochemical characterization of the nuclear antigens involved in the GS-ANA reaction has been precluded by the scarcity of methods available for their detection. Thus the spontaneous antibodies occurring in sera of RA patients have been shown to be demonstrable only by indirect immunofluorescence or immunoenzyme technique. Repeated attempts at showing precipitating activity of GS-ANA with granulocyte nuclear extract have given completely negative results (219). Complement fixation and passive haemagglutination techniques have been considered inappropriate since only subsets of antibodies can be disclosed by these methods. Detection of soluble nuclear antigens of granulocytes has been attempted by inhibition of indirect immunofluorescence using a battery of standard GS-ANA sera from RA patients, but the technique proved to have little value due to low sensitivity and poor reproducibility (219).

The heterogeneity of nuclear antigens involved in the GS-ANA reaction is indicated by the occurrence of very different but remarkably reproducible staining patterns exhibited by individual patient sera employing a standard technique (see section 3) and fixed dilutions of test sera and conjugates (220). Conjugates specific for the different human Ig heavy chains often revealed different distinct patterns which coalesced into composite patterns when a polyspecific anti-Ig conjugate was used, indicating heterogeneity of the GS-ANA reaction in individual sera. Such variations have even been noticed within the IgG subclasses of the antibodies (227).

Partial characterization of granulocyte-specific nuclear antigens have been attempted by enzyme treatment of the leucocyte smears used as substrate for the ANA technique (50, 56). From such studies it was proposed that DNA was likely to constitute part of the antigens, since DNA-ase treatment eradicated the reactivity with most GS-ANA positive sera. However, control treatment with phosphate buffered saline (PBS) alone resulted in removal of antigens in some cases (56), indicating high solubility of these antigens in PBS. Studies taking the pronounced solubility of nuclear antigens into account by postponing the enzyme treatment till after the reaction with patient serum had taken place (209) showed a preserved though pattern-wise altered nuclear fluorescence with most sera (220). Control studies using PBS treatment interposed in the same part of the staining procedure resulted in a completely retained reactivity of all sera, while PBS treatment before serum application indicated involvement of saline soluble as well as saline insoluble nuclear antigens in the reaction (220). RNA-ase treatment did not produce negative reactions.

The basic question pertaining to the solubility of granulocyte specific nuclear antigens in the most widely used buffer for

immunofluorescence work, PBS, seems not to have been emphasized previously, despite the fact that numerous investigators have utilized human peripheral blood leucocytes as a convenient nuclear substrate for ANA detection, these cells still being the preferred substrate for this purpose in many laboratories. It is possibly the main factor responsible for the great discrepancies reported on the prevalence of ANA in RA (reviewed in 50) and the key to the question why publications on GS-ANA have been rather scarce (18, 41, 47-57, 65, 79, 93, 96, 97, 109, 130, 144-149, 161, 164, 173, 184, 193, 198, 202, 204, 205, 211, 215-229, 242, 248) originating from a rather low number of laboratories, all of which employed the leucocyte substrate after some form of fixation or prolonged air drying followed by direct application of test serum without prewashing the smears.

Recent data from our laboratory obtained by enzymatic treatments of the leucocyte nuclear substrate in a low ionic strength-phosphate buffer without saline to minimize nuclear antigen extraction during the treatment (Table 1) support the concept that DNA and RNA are not major parts of the granulocyte-specific nuclear antigens involved in the reactions with most RA patient sera. The eradication of reactivity upon trypsin treatment in all cases indicates that proteins are the most essential antigenic constituents. The extinction or pronounced reduction of reactivity upon treatment of leucocytes with periodate (Table 1) may indicate a glycoprotein nature of some of the specific nuclear antigens of granulocytes. The high solubility of the antigens in PBS was confirmed and extended by using 0.1 M HCl treatment before a short PBS equilibration and serum application to mirror the technique used by some investigators (230, 231) who also failed to show GS-ANA. The high solubility of the antigens parallels a number of more recently described soluble nuclear antigens responsible for reactions with ANA in systemic lupus erythematosus, mixed connective tissue disease, polymyositis, Sjögren's syndrome and RA

Table 1

Reactivity of twenty-three rheumatoid arthritis patient sera containing IgG GS-ANA with human granulocyte nuclei after treatment of these with various enzymes and chemicals

Treatment of nuclear substrate	No. of pos./ total no.
Untreated	23/23
Phosphate buffer (0.01 M)	
with $MgCl_2$ (0.003 M) (PBMg)	22/23
Phosphate buffered saline (PBS)	11/23
Trypsin (0.1 mg/ml) in PBMg	0/23
DNA-ase (1 mg/ml) in PBMg	16/23
DNA-ase (1 mg/ml) in PBMg after patient serum (206)	17/23
RNA-ase (1 mg/ml) in PBMg	22/23
Sodium periodate 0.1 M in PBMg	18/23
HCl 0.1 M	14/23

Table 2

Titres of ANA reacting with nuclei of human neutrophilic granulocytes (N), lymphocytes (L) and rat liver cells (R) in sera of rabbits immunized with human granulocyte nuclei (rabbits, 499-503) and human lymphocyte nuclei (rabbits, 504-508)

Bleeding no.: Nuclear reactivity:	I*			II*			III*			IV*			V*			VI*		
	N	L	R	N	L	R	N	L	R	N	L	R	N	L	R	N	L	R
Rabbit																		
499	1024	16	<16	512	<16	<16	8192	<16	<16	8192	<16	<16	16384	<16	<16	4096	<16	<16
500	4096	<16	16	4096	<16	16	16384	<16	<16	8192	<16	<16	16384	<16	<16	4096	<16	32
501	4096	<16	<16	4096	16	<16	16384	16	<16	4096	<16	<16	4096	<16	<16	nt.	nt.	nt.
502	2048	<16	<16	2048	16	<16	4096	<16	<16	2048	<16	<16	2048	<16	<16	2048	<16	<16
503	1024	<16	64	1024	16	32	4096	<16	16	2048	<16	64	nt.	nt.	nt.	2048	<16	64
504	<16	<16	<16	<16	32	<16	<16	<16	<16	nt.	nt.	nt.	nt.	nt.	nt.	nt.	nt.	nt.
505	16	16	16	<16	16	<16	<16	16	<16	<16	16	<16	<16	<16	<16	<16	<16	<16
506	<16	<16	<16	<16	32	<16	<16	32	<16	<16	16	<16	<16	16	<16	<16	16	<16
507	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
508	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16	nt.	nt.	nt.	<16	16	<16

* Intervals between initiation of immunization and bleeding: I, 2 months; II, 3 months; III, 4 months; IV, 7 months; V, 11 months; VI, 14 months.
nt. = not tested.

(4-5, 62, 89, 117, 128, 135, 156, 172, 187, 207, 234). Some of these are extracted so easily that no fixation or prewashing is permissible if reactivity is to be preserved (4-5).

Early studies by Barnett & Vaughan (18) indicated presence of granulocyte specific nuclear antigens in most normal human sera since rabbits immunized with such sera developed GS-ANA. The simplest explanation for this phenomenon would be that nuclear antigens are extruded from granulocytes during blood clotting, but confirmation of this possibility was not sought. The fact that normal sera often inhibit the GS-ANA reactions of RA sera seems to support this notion (219).

A pronounced immunogenicity of granulocyte nuclei when injected into experimental animals has been shown by several investigators (109, 127, 226, 242). A better response was obtained when whole nuclei preincubated with ANA were used as the immunogen (242). Our own experiments utilized citric acid/sucrose treatment of purified human granulocytes, and isolation of the nuclei by differential centrifugation in sucrose to avoid extraction of soluble nuclear antigens. The nuclei were injected into New Zealand white rabbits using both subcutaneous administration in Freund's incomplete adjuvant and intravenous injections in saline alternately every second week. GS-ANA were readily detectable by indirect immunofluorescence after 2 months of immunization, and continued immunization led to production of high titered GS-ANA (Table 2). Similarly obtained lymphocyte nuclei injected into control rabbits in the same manner resulted in little or no specific ANA production. The combined data indicate presence of powerful immunogens in the saline extractable part of human granulocyte nuclei. The apparent difference in immunogenicity may be related to the observation made previously that GS-ANA appear before ON-ANA in sera of NZB/W mice (50), in thymectomized Swiss mice (193) as well as in RA (50) and in very early phases of systemic lupus erythematosus (219).

Recent data indicate that DNA-associated, non-histone proteins may be responsible for the specificity of experimentally induced GS-ANA (109). These antigens may well contribute substantially to GS-ANA reactions of RA sera as judged from the data discussed above.

3. TECHNICAL ASPECTS OF GS-ANA DETERMINATION

The original study of Faber et al. (57) and all subsequent studies by these investigators (reviewed in 50, 54) utilized repeatedly frozen and thawed smears of freshly obtained blood from normal donors as cellular substrate for the GS-ANA detection. This technique is attractive with regard to preservation of maximum reactivity of the nuclear antigens (50) and rendered the nuclei satisfactorily accessible to reactions with GS-ANA (49). Inherent draw-backs of the technique were the lack of fixation of the cells to the slide surface causing loss of a great number of lymphocytes during washing manipulations, but also the undesirably strong background fluorescence due to serum Ig adsorbed to the slide and cell surfaces and an inconveniently low number of cells per microscopic field for quick reading of results in a daily routine.

After weighing various advantages and drawbacks against each other a technique was developed which used isolated normal donor buffy coat cells from defibrinated blood, obtained by flotation on a dextran-Isopaque mixture at 1 x g for 45 min at room temperature (227). After washing in PBS containing 1% human serum albumin the cells were smeared onto defatted glass slides and fixed in 4°C cold ethanol for 5 min. Ethanol has been the fixative of choice for leucocytes used by many investigators (16, 28, 42, 70, 79, 173, 185) and our initial studies found it superior to acetone, methanol and para-formaldehyde with regard to maximum preservation at nuclear antigen reactivity and fluorescence pattern. None of the fixatives were able to insolubilize the PBS extractable nuclear antigens of granulocytes discussed in

section 2. Unfixed cryostat sections 4–5 μ m thick of human thyroid or rat liver were always applied to the same slide, and no washing was done prior to test serum application. Pilot studies of 100 sera were run by the technique of Faber et al. (57) and by the novel technique, henceforth designated the standard technique. These sera were derived from a large routine laboratory, the Laboratory of Autoimmune Serology at Statens Serum Institute, Copenhagen. Results of ANA screening were unknown to us. We used the identical conjugate with specificity for all three main Ig classes. One serum out of 60 shown to be positive for GS-ANA by the freeze-thaw technique was negative by the standard technique, the opposite being seen in two sera, illustrating the good overall concordance of the two methods (222).

This standard technique has been used in all subsequent studies of GS-ANA, the details of which have all been published (191, 224, 227, 228).

The use of the standard technique for IgG GS-ANA and ON-ANA demonstration yielded reproducible results from day to day showing standard deviations of less than one dilution step by titration of the same sera on 10 consecutive working days. The technique proved reliable, easy to perform, easy to read and well suited for detailed specific antibody characterization (see sections 5 and 6).

4. OCCURRENCE OF GS-ANA IN HEALTHY PERSONS

Studies on ANA in various diseases have to be based on control investigations of healthy persons to define the borderline which for practical purposes has to be set between normal and abnormal concentrations of the antibodies. Such studies are also warranted from a theoretical viewpoint since it is not generally established whether ANA should be considered normal or abnormal constituents of human serum. Surprisingly few studies of ANA in health and disease have dealt with the influence of age and sex on the prevalence and

levels of ANA in serum, and even technical variables such as nature of nuclear substrate, dilution of test serum, specificity of conjugate, etc., are often left out. Although comparisons thus cannot be made between data of different reports, it seems to be established that a variable proportion of apparently healthy persons do have circulating ANA (reviewed in 50 and 62).

GS-ANA have been looked for in normal individuals in few studies (49, 50, 130, 144, 161, 202, 218, 224). The prevalence reported has varied from 0 to 30%, depending on selection of donors, method, serum dilution and Ig class of GS-ANA looked for. In 466 randomly selected sera from 20–60-year-old normal blood donors Wiik (218) found ANA of one or more Ig classes in 77% of undiluted sera, IgM, IgA and IgG ANA being detected in 53%, 44% and 14%, respectively. ANA reacting solely with phagocyte nuclei constituted about one fourth of these normally occurring ANA. Dilution of sera to 1:16 resulted in a decrease in all positive reactions. IgM, IgA and IgG GS-ANA were now found in 8%, 3% and 1%, respectively. These prevalences obviously represent minimum values since no absorption of the sera was done to remove ON-ANA. These data tend to recommend the use of both qualitative and quantitative measures for ANA in routine serology. Our laboratory has chosen to study routine sera only for IgG class ANA using a screening dilution of test serum of 1:16. Women above 40 years of age showed an increased prevalence of IgG ANA (218), but otherwise only minor differences were noted among the age and sex groups studied. Low concentrations of ANA have been demonstrated also in sera from normal persons of both sexes between 0 and 20 years of age (219), and in dilution 1:16 the prevalences of ANA of the three main Ig classes are roughly similar to those observed in adults (144). In normal sera neither GS-ANA nor ON-ANA were found to possess CF properties (218, 224) suggesting little or no phlogogenicity of these ANA which may be serving a physiological purpose or have a protective role (171) as has

been suggested for rheumatoid factors (RF) in healthy persons (89, 99).

After the age of 60 years the prevalence and titres of ANA increase (29, 186, 231). This has been confirmed by Wiik et al. (224) who found IgM, IgA and IgG ANA in a titer of 1:16 in normal control sera of 64, 36 and 20%, respectively. GS-ANA of the IgM and IgG classes constituted 20% and 8%, respectively. Whether these data reflect a gradual loss of self tolerance or simply an enhanced physiological mechanism triggered by increased tissue catabolism is not known, but it should inspire to caution in interpreting ANA seropositivity in diseases commonly encountered in older people such as rheumatoid arthritis.

The existence of GS-ANA in normal control persons at all ages and the heterogeneity of these antibodies in disease (see section 5) argues against a simple clonal selection or somatic mutation theory at the level of the B lymphocyte itself. The data rather indicate that GS-ANA are products of a qualitatively normal immune response which may be triggered to a broad polyclonal antibody production in patients with rheumatoid arthritis and especially in patients with Fely's syndrome (see sections 5 and 8).

5. Ig CLASS AND IgG SUBCLASS NATURE OF GS-ANA IN RA

The first patient described to have GS-ANA (57) suffered from the classic Fely's syndrome (61). The specific antibody was estimated to be of IgM class due to sulphydryl sensitivity and macroglobulin sedimentation characteristics upon ultracentrifugation analysis. Subsequent studies showed that GS-ANA were commonly found also in other forms of RA (48, 52, 53, 56) and the antibodies belonged to the IgG, IgA and IgM classes (49). Studies of granulocyte-reactive ANA, which comprise GS-ANA in the presence or absence of ON-ANA, have disclosed a similar Ig class heterogeneity of the antibodies (13, 16, 51, 53, 97, 130, 202).

By the use of the standard technique Wiik et al. have confirmed the heterogeneity of GS-

ANA in RA sera (148, 220, 224, 227-229). The combined data indicate that the antibodies first and foremost occur in the IgG and IgM classes, though IgA, IgD and IgE class GS-ANA may also be found. These latter Ig classes of GS-ANA are especially frequent in sera from neutropenic cases of RA who also characteristically have very high titres of IgG and IgM GS-ANA. The IgG subclass composition of GS-ANA in individual sera differs considerably (227). The antibodies may belong to one, two, three or all four subclasses of IgG, CF properties being closely related to the IgG₁ and/or IgG₃ subclasses of the antibodies. The preponderance of ANA in the IgM class pointed out in early studies of RA sera (209, 238) is thus not reflected in the GS-ANA system where IgG antibodies are predominant both in adult RA (47, 184, 224, 227, 228) and in juvenile chronic arthritis (97, 130, 144, 161).

The apparent heterogeneity of GS-ANA both as regards nuclear antigen specificity and Ig class and subclass composition makes these antibodies especially interesting since they compare well with details reported on ON-ANA in systemic lupus erythematosus where ANA are ascribed a definite role in the pathogenesis of the disease (62, 91, 101, 105, 106, 110, 143, 153). In this disease CF properties are similarly linked to the IgG₁ and/or IgG₃ subclasses of the antibodies (101, 110, 153, 169, 176, 193) and a high CF potential of ANA is related to a more severe course of the disease (139, 153, 170, 176, 194).

At this point it should be mentioned that an unambiguous Ig class and subclass characterization of any particular antibody in unfractionated serum of RA patients is hampered by the coexistence of RF, immunoglobulins and probably other auto-antibodies which may simulate the antibody looked for by binding secondarily to the specific antigen-antibody complex formed (16, 118). Similar masking of GS-ANA probably by RF has been noted in studies by Wiik (216, 217) where sera or joint fluids had been fractionated by gel filtration at acid pH

(discussed in sections 9 and 10). The fact that GS-ANA commonly participate in immune complexes of RA sera and joint fluids (see sections 7, 9 and 10) may also influence the attempts at characterizing the antibodies in detail.

In summary the standard technique used by Wiik and others (97, 130, 161, 184) thus produces results similar to those of the freeze-thaw technique (47, 50), imposing no restriction on the detectability of the various Ig classes of GS-ANA.

6. COMPLEMENT-FIXING PROPERTIES OF GS-ANA IN RA

In most cases of RA showing presence of GS-ANA in serum and/or joint fluid the antibodies lack CF properties (224, 227, 228). However, when the disease is complicated by neutropenia the GS-ANA as a rule exhibit various degrees of CF abilities (228), high titres of CF GS-ANA being present solely in active phases of the disease with manifest neutropenia whether accompanied by splenomegaly or not. Normalization of neutrophil concentrations accomplished by corticosteroid treatment is accompanied by a conspicuous fall in titre of CF GS-ANA (219). It is most probable that it is the IgG class of GS-ANA which governs the CF abilities of the antibodies, firstly since this property is so closely linked to the IgG subclass nature of the antibody described above, and secondly since CF GS-ANA always appear in IgG containing fractions upon Sephadex G-200 gel filtration (216, 217).

CF properties of GS-ANA are likely to furnish them with enhanced phlogistic capabilities and the relation demonstrated in RA to neutropenia may indicate some mechanism by which these antibodies contribute to this rather rare complication of RA (see section 9).

7. SYNOVIAL MEMBRANE SYNTHESIS OF GS-ANA IN RA

No direct evidence has been produced for the notion that ANA may be synthesized locally

by the synovial membrane in RA since direct investigations of such production have not been undertaken. Still some investigators seem to assume that such antibodies may be synthesized locally since ANA is detectable in synovium eluates when undetectable in the corresponding joint fluid and serum (35), and since ANA may be found in joint fluid and not in the corresponding serum (14, 53, 69, 114, 130).

The same line of reasoning stimulated studies of paired RA joint fluids and sera for the occurrence and titres of ON-ANA and GS-ANA belonging to the three main Ig classes (224). Most joint fluids contained titres equal to or lower than those of the paired serum, but some cases showed higher titres of ON-ANA or GS-ANA in joint fluid than in serum.

This was found for all of the three main Ig classes studied. However, such data should be interpreted with great caution since a number of factors are likely to influence the results:

- (a) The rheumatoid joint fluid is known to contain immune complexes of varying size and composition (36, 82, 112, 116, 122, 124, 232, 233, 246). The emerging evidence of participation of GS-ANA in such complexes makes comparison with serum titres problematical, as most GS-ANA activity in RA serum seems to be uncomplexed (216).
- (b) Immune complexes are constantly being removed from the joint fluid by phagocytosis by neutrophils and monocytic cells and by type A and C cells of the lining layer (3, 23, 24, 90, 94, 104, 115, 154, 248). Antibodies participating in such complexes may therefore to some extent evade detection.
- (c) Nuclear antigens – both soluble and insoluble – have been demonstrated to be richly represented in RA synovial fluids (12, 115, 160). These antigens may block the ANA of joint fluid partly or completely. This concept has found support from joint fluid GS-ANA fractionation studies (217). Comparable concentrations

- of nuclear antigen in serum are probably not found (12, 188).
- (d) The majority of RA sera contain rheumatoid factors and immunoglobulins as well as various IgG agglutinators which are likely to bind secondarily to IgG and complement, respectively, whereby masking the Ig class and content of ANA in an unpredictable way (see sections 9 and 10).
 - (e) Autoantibody affinity at the level of the producing plasma cell or lymphocyte may be quite different from that of the circulating autoantibody (158) favouring sequestration of high affinity antibody in immune complexes adjacent to the site of production.
 - (f) Lysosomal proteinases are abundantly present in rheumatoid joint fluids (reviewed in 3, 213, 246). If these enzymes are not totally inhibited by corresponding proteinase inhibitors joint fluid Ig may be degraded (58).

Most of the mentioned factors are likely to influence the quantity and the quality of ANA demonstrable by studies of unfractionated joint fluid samples, while only few of the factors are likely to affect the antibodies demonstrable in serum. The values recorded in joint fluids thus probably represent minimum values. Nevertheless, a definite proof of local synthesis of GS-ANA and ON-ANA must await future direct studies of antibody synthesis.

8. CLINICAL SIGNIFICANCE OF GS-ANA DETERMINATION

Already from the early start of GS-ANA serology it became evident that these antibodies could be considered as characteristic of RA, as were the RF (52, 54, 56) although their presence is not limited to this disease (56, 96, 149, 164, 173, 198, 202, 218). Still GS-ANA belonging to the IgG class are more common in RA than in any other disease entity, and in Felty's syndrome they are only rarely absent (56, 228). These antibodies may be found in

the acute phase of drug-induced lupus erythematosus (56, 149, 198) and even in other drug-related syndromes (149). GS-ANA are sometimes found in spontaneous systemic lupus erythematosus (47, 56, 173, 243) though in most of these patients they are masked by the simultaneously occurring ON-ANA which therefore must be removed by absorption to uncover the usually low-titered GS-ANA (47). Interestingly the systemic lupus erythematosus patients who exhibited GS-ANA in the latter study were those that had the most severe complaints of arthritis as part of their disease pattern. In the early phase of the NZB/W disease of mice GS-ANA have been found to appear before ON-ANA (50). Correspondingly, a few patients investigated for ANA in a very early phase of systemic lupus erythematosus have been found to produce high titres of IgG GS-ANA with pronounced CF properties (219). These antibodies appeared some weeks before ON-ANA, which eventually hid the GS-ANA. GS-ANA may be sporadically found in patients with chronic active hepatitis (149, 173), myasthenia gravis (173) and chronic ulcerative colitis (149). GS-ANA are also found in juvenile chronic polyarthritis (130, 144, 149, 161), but there is some disagreement as to the prevalence, some finding these antibodies commonly in several clinical types of the disease (130, 161) and others predominantly showing them in girls having many affected joints (144, 149).

Earlier studies of RA patients have shown a correspondence between disease activity as judged by the presence of anaemia, a raised erythrocyte sedimentation rate and severely compromised locomotor function and the presence of ANA detected by the use of human leucocytes as nuclear substrate (2, 31). However, these data are at variance with those of Elling & Graudal (51) who found no relation to the stage of the disease, the degree of anaemia or joint functional capacity.

Very recent data have shown a close correlation between presence of IgG GS-ANA in serum and progression of joint damage both at the level of single affected joints and in the

number of affected joints of adult RA patients followed for at least 3 years by a graded radiographical technique (40). No such relation was found for IgG ON-ANA. IgG GS-ANA may have similar prognostic significance in juvenile chronic arthritis since propagation of disease activity into adult age is accompanied by presence of these antibodies in the patients sera (45).

If ANA are semiquantitated by titration the diagnostic value of the test is increased considerably (2, 55, 62, 111, 138, 149, 157, 207). If CF properties are also estimated by titration still more information of importance for the clinician is obtained (34, 139, 149, 153). Similarly a titration of IgG and CF GS-ANA may enhance the value of a positive test appreciably, high titres of IgG GS-ANA with CF abilities being limited to RA patients having accompanying neutropenia or patients with a rheumatoid factor positive autoimmune neutropenia lacking arthritis (228), though low titres of CF GS-ANA may be seen even in uncomplicated RA, especially in patients having a positive LE cell phenomenon and in patients with drug-induced lupus erythematosus.

IgD and IgE class GS-ANA are significantly more often found in RA patients with accompanying neutropenia (154, 229) than in uncomplicated cases of RA. Determination of these minor Ig classes of GS-ANA may turn out to be of aid in the differential diagnosis between autoimmune neutropenia of RA versus other forms of neutropenia in RA patients as indicated also for CF GS-ANA (228).

9. PARTICIPATION OF GS-ANA IN CIRCULATING IMMUNE COMPLEXES IN RA

Many studies of RA sera have indicated the existence of circulating immune complexes *in vivo*. Presence of IgG aggregates of molecular sizes above that of monomeric IgG has been shown by ultracentrifugation (6, 63, 92, 107, 134, 167, 233) and by Sephadex G-200 gel

filtration (19, 134, 159). Others have used precipitation of such aggregates with IgM rheumatoid factors (82, 233), radioimmunoassay with isolated monoclonal IgM rheumatoid factor (112), aggregation of platelets (134), cryoprecipitation of serum (78, 93, 210, 211), histamine release from guinea pig lung (19, 75), and phagocytosis by neutrophilic granulocytes (6, 78, 93).

Most of the methods mentioned have essential limitations: (a) since it is not possible to perform the techniques under conditions by which immune complexes are dissociated—a step which is desirable to be able to discriminate immune complexes from artificial aggregates arising from inappropriate handling of the serum sample; (b) since analysis of antigens and specificities of the antibodies involved in aggregate formation is not possible.

Wiik (216), therefore, chose to analyse RA sera for the existence and nature of immune complexes by subjecting them to Sephadex G-200 gel filtration at neutral pH, the advantage being that sufficient amounts of material were obtained in each fraction for multiple analyses of its specific antibody content and the Ig class nature of these antibodies. Titration of the various Ig classes of GS-ANA, ON-ANA and normally occurring heterophilic antibodies to rabbit red cells allowed the estimation of a selective versus an indiscriminate involvement of these antibodies in high and intermediate size aggregates, the former being interpreted in favour of an immune complex nature of the aggregate, the latter of an artificial aggregation. This technique has the additional advantage that fractionation may be carried out at acid pH which is expected to cause dissociation of immune complexes (82, 107, 167, 233) though admittedly some artificial aggregates may, similarly, disaggregate to a certain extent at acid conditions (84).

Until recently RF belonging to the IgG and IgM classes have been the only specific antibodies recognized in circulating immune complexes in RA (82, 107, 167, 233). Some investigators seem to imply that nearly all specific antibody activity contained in inter-

mediate complexes in RA may be ascribed to self-associating IgG RF (113, 151, 152, 239) even if careful analyses of such complexes have been performed only in a few patients displaying a very rare disease picture accompanied by hyperviscosity of the blood (151). Polyclonal IgG and IgM RF contained in intermediate and high molecular size complexes of patients with RA have recently been shown to cross-react with cell nuclei from many species, i.e. these antibodies also have ON-ANA activity (82, 100). Apart from this, very little has been done to explore whether other antibody specificities might be involved in immune complex formation in the majority of patients with RA.

The wide-spread occurrence of GS-ANA in active RA (52, 53, 224), the appearance of these antibodies early in the disease (50-52), and the possible pathogenetic significance of these antibodies (14, 245, 248) made GS-ANA interesting targets for investigation of immune complexes.

Sera from patients with Felty's syndrome, neutropenic cases of RA, non-neutropenic cases of RA, osteoarthritis and normal control persons were fractionated on a Sephadex G-200 column at neutral pH in Tris-HCl buffer, and all fractions were studied for the presence of IgG, IgM and IgA ON-ANA and GS-ANA, and IgG, IgM and IgA rabbit red cell antibodies by indirect immunofluorescence technique as well as for RF by latex agglutination technique (216). A very clear-cut picture emerged. IgG and IgA GS-ANA as a rule were eluted in fractions containing high and/or intermediate size proteins, i.e. in fractions of the first peak and the area between peak I and peak II indicating some form of aggregation of these antibodies. In contrast, IgG and IgA rabbit red cell antibodies mostly appeared in the fractions of the second peak. The quantity of IgG GS-ANA involved in aggregates was especially high in sera from neutropenic RA patients where complement fixability of the GS-ANA was similarly extended to both peak I and II fractions as well as the interval

between these. As much as 50% of the total IgG GS-ANA titer units could be found in aggregate containing fractions in sera of Felty patients.

Upon fractionation of sera at pH 4.5, IgG, IgA and CF GS-ANA as a rule were eluted in peak II fractions indicating dissociation of immune complexes involving these antibodies. Complement fixability of GS-ANA was most probably associated with the IgG class of the antibodies, similar to what has been shown for CF ON-ANA (101, 153, 169, 176, 194, 195). When present, IgG and IgA ON-ANA behaved closely similar to GS-ANA at neutral and acid pH. RF activity of two thirds of the sero-positive sera was found both in 19S and 7S containing fractions, indicating varying molecular sizes of these antibodies.

An unexpected finding was the appearance of IgM class GS-ANA or ON-ANA not only in the 19S region of the elution profile but also in 80% of the RA sera in peak II fractions, indicating a monomeric form of these antibodies not earlier described in RA. Disappearance of these antibodies from some sera after fractionation at pH 4.5 suggests pronounced sensitivity of monomeric IgM antibody activity to even moderately low pH conditions. With only two exceptions IgM rabbit red cell antibodies were restricted to the 19S peak.

An interesting observation was made on serum from a Felty patient which showed strongly positive reactions for IgG, IgM and IgA GS-ANA after gel filtration at neutral pH in spite of the fact that the whole serum and both earlier and later whole serum specimens had been shown to be negative for all of these antibodies by repeated examinations. This points towards removal of GS-ANA inhibitors—possibly nuclear antigens—from both peak I and II fractions as a result of the gel filtration. Two sera shown to give negative RF reactions disclosed such activity in 19S fractions upon gel filtration at neutral pH, suggesting the removal of agglutination inhibitors (21, 82). IgM ANA seen in fractions of a Felty patient serum disappeared completely upon acid fractionation, possibly indicating

IgM RF simulating ANA by complex formation (13, 118).

Altogether the study indicates a regular participation of GS-ANA but not rabbit red cell antibodies in aggregates which appear to be immune complexes, IgG GS-ANA being especially enriched in complexes detected in neutropenic cases of RA where the antibodies show complement fixability. Low molecular size IgM GS-ANA and ON-ANA were prevalent in RA patients having accompanying neutropenia, thus confirming and extending earlier observations that 7S IgM is found predominantly in RA patients having extraarticular manifestations (92, 177, 192). The 19S state of IgM antibodies which bear no relation to the rheumatoid inflammation (i.e. rabbit red cell antibodies in RA and control sera, and ANA in normal control sera) indicates an as yet unexplained relation between low molecular size IgM autoantibodies (GS-ANA and RF) and the rheumatoid inflammation. The involvement of several autoantibodies in immune complexes in RA, the existence of antibodies capable of binding secondarily to an immune complex and the presence in some sera of ANA and RF inhibitors make any analysis of Ig class and content of specific antibody inexact (see section 7). A recent report confirms the predominance of granulocyte-reactive ANA in circulating cold-insoluble immune complexes isolated from Felty's syndrome patients (211). The antibodies appeared to be selectively concentrated in these complexes.

IgD and IgE class GS-ANA found in neutropenic cases of RA also seem to be involved in circulating immune complexes as shown by molecular sieving and dissociation experiments and cryoglobulin studies (148, 229).

10. PARTICIPATION OF GS-ANA IN JOINT FLUID IMMUNE COMPLEXES IN RA

Two approaches have been used to elucidate the question whether or not GS-ANA may

participate in immune complex formation in the rheumatoid joint fluid. At temperatures well below those encountered in the circulating blood certain immune complexes become insoluble. Though no equation mark can be put between these so-called cryoglobulins and immune complexes the latter seem to be major constituents of cryoglobulins isolated from sera and joint fluids in diseases where immune complexes are prevalent and held to be significant for the pathogenesis of inflammation (15, 116, 246). Earlier studies of RA synovial fluids have indicated presence of significant levels of cryoglobulins in most cases of sero-positive disease (15, 36, 38, 116, 196, 246). Cryoprecipitable material was responsible for most of the CF capacity found in RA joint fluids (116, 196), and an inverse relation was shown to the complement level of the fluid from which the cryoglobulin originated (246). The cryoglobulins consisted of IgG, IgM, various complement components, denatured probably single stranded DNA and some fibrinogen antigenic material (36, 116, 246).

Wiik et al. (225) isolated cryoproteins from 42 RA and 18 osteoarthritis joint fluids and found the former richer in total protein, Ig and complement components. However, this could be a mere reflection of higher concentrations of these constituents in the parent joint fluids. In contrast, IgG and/or IgM GS-ANA were demonstrated in 62% of the RA and in none of the control cryoproteins, and a comparison of the relative amounts of IgG GS-ANA in paired cryoproteins/synovial fluids indicated enrichment of rheumatoid cryoproteins with these particular antibodies. IgG ON-ANA appeared to be enriched in a similar way though found less commonly. Also RF and immunoconglutinin activity was associated with RA joint fluid cryoproteins. These data imply that GS-ANA as well as ON-ANA, RF and immunoconglutinins participate in cold-insoluble immune complexes of rheumatoid joint fluids.

This notion was further supported by data from Sephadex G-200 fractionation of RA

joint fluids at neutral and acid pH (217). Most of the RA joint fluids contained IgG GS-ANA which always eluted in fractions containing high and intermediate molecular size proteins at neutral pH. In contrast, IgG rabbit red cell antibodies as a rule were present in 7S rich fractions only, pointing towards aggregation of IgG GS-ANA in preference to IgG heterophilic antibodies, most likely reflecting an immune complex constitution of the aggregates. Upon fractionation at pH 4.5 most of the IgG ANA activity was displaced to peak II fractions indicating partial and sometimes complete dissociation of the complexes. The subtotal dissociation under these experimental conditions compared to sera of the aggregates may denote a higher affinity of the involved antibodies in the joint fluid. This may be a pendant to the higher affinity of locally produced RF than of circulating RF shown recently (158). A higher affinity of IgG GS-ANA than of IgA and IgM molecules simulating GS-ANA activity but probably possessing RF specificity was indicated by the persistence of high molecular size IgG GS-ANA contrasting with the disappearance of IgM and IgA molecules of apparent GS-ANA specificity upon gel filtration at pH 4.5. Removal of GS-ANA inhibitors at neutral pH gel filtration was shown in one case. Low molecular size IgM ANA were present in most RA joint fluids.

11. THEORIES CONCERNING THE IMMUNOPATHOGENESIS OF RA AND THE POSSIBLE PATHOGENETIC SIGNIFICANCE OF AUTOANTIBODIES WITH SPECIAL REFERENCE TO GS-ANA

The pathology of the rheumatoid synovium is similar to that of an active lymphoid organ being built up by a richly vascularized loose connective tissue stroma, an abundance of small lymphocytes often organized into follicle-like structures, surrounded by plasma cells and mononuclear phagocytic cells. The lining layer is characterized by hypertrophy

and hyperplasia dominated by phagocytic type A and C cells mixed with hyaluronate producing type B cells. Granulation tissue in the form of pannus is typically found along the articular cartilage, and at the boundary of the pannus cartilage destruction is seen. The joint fluid is hypoviscous containing large numbers of inflammatory cells the majority of which are neutrophilic granulocytes (for review see 81, 246).

Several discoveries in the late fifties and early sixties led to the proposition that immunological phenomena were essential for the pathogenesis of rheumatoid synovitis: (a) the plasma cells of the synovium were found to produce IgG, IgM and RF (102, 123); (b) IgG, IgM and complement was found in and around the vessel walls and in amorphous deposits in the interstitium (102); (c) the phagocytic cells of the lining layer and the synovial fluid were shown to contain cytoplasmic inclusions consisting of IgG, IgM and complement (90, 154); (d) the level of haemolytic complement in RA joint fluids was lower than that of non-inflammatory joint fluids (87, 142); and (e) lysosomal enzymes were richly present in RA joint fluids (98, 175).

Scientists in the field tried to encompass these data into a hypothesis on the pathogenesis of RA (90, 238, 245). They all held the joint an ideal site for local antibody production triggered off by an unknown inciting agent reaching the joint. The ensuing association of this antigen with specific antibody in the synovial membrane or fluid into immune complexes would activate and consume complement giving rise to anaphylatoxin and chemotactic factor formation from activated complement. Both types of mediators would alleviate diapedesis and attraction of neutrophilic granulocytes from the synovial membrane vessels to the joint fluid where these cells would ingest the complement-armed immune complexes. However, in doing so the granulocytes would leak out lysosomal enzymes capable of causing tissue damage. The basis for the perpetuated inflammation could be some

form of inflammation-induced alteration of self constituents conferring immunogenicity to these and hence resulting in autoantibody production and continued immune complex formation (90, 212).

A large body of evidence in support of this basic concept has emerged from detailed studies of each step in the vicious cycle of reactions mentioned, and immune complex mediated mechanisms of inflammation are still held responsible for the perpetuated synovitis leading to erosive changes of cartilage and bone (246). The main emphasis of the present review is put on this basic immunopathogenic model since it is of prime relevance for the GS ANA studies referred to and since the most solid evidence has been presented for its actual operation in the rheumatoid patient. On the other hand there can be little doubt that cell mediated immune reactions have a role in rheumatoid synovitis as well (1, 7, 11, 43, 75, 77, 103, 163, 178, 200, 236, 240, 247). However, since no data point to direct T lymphocyte mediated tissue damage occurring in the joint, the main role of T lymphocytes could be the regulation of B lymphocyte activity and thus antibody production (247). From studies of experimentally induced arthritides in animals there is ample evidence that a cellular immune response towards the immunogen must be evoked if a chronic erosive synovitis is to be maintained (7, 43, 73, 236). A similar situation may well exist in RA since removal of T lymphocytes from RA patients often leads to abrogation of disease activity even in very severe cases (140, 208, 236). Still agammaglobulinaemia patients contracting chronic arthritis have a mild disease resulting in little or no permanent tissue damage (76) indicating only a modest role of T lymphocyte function in the absence of normal B lymphocyte function.

Special attention has recently been called to the fact that intraarticular immune complexes tend to localize in cartilagenous tissues such as menisci, ligaments and cartilage (32, 33). This type of sequestration may have importance for the presumed final pathway of tissue damage

since neutrophilic granulocytes confronted with non-phagocytosable immune complexes turn into potent lysosomal enzyme secretory cells (reviewed in 88). The main cell type responsible for cartilage destruction, however, seems to be mononuclear phagocytic cell, which is likewise inducible to lysosomal enzyme release upon contact with immune complexes (reviewed in 3). A higher affinity of locally produced than of circulating RF tends to sequester the former in the joint (131, 158). Similar characteristics may apply to other locally produced antibodies and thus have relevance for the confinement of most inflammatory activity to the joints.

Several other biological effects have been postulated for immune complexes: A blastogenic effect on lymphocytes in the presence of complement (103), a leucocyte migration inhibitory factor inducing effect on lymphocytes (25, 66), a macrophage migration inhibitory factor inducing effect (46), an immunoglobulin synthesis enhancing effect when bound to IgM RF with complement (235), a K cell inhibitory effect on antibody dependent cellular cytotoxicity (80, 159), and a neutrophil chemotaxis and phagocytosis inhibitory effect (129, 159, 197). The actual biological significance of all these potential mechanisms *in vivo* is unknown, but it is conceivable that they can account for at least some of the lymphokine/monokine-like activity demonstrated in RA joint fluids (163, 178) and possibly for the compromised defence against bacterial infections encountered in some patients (78, 92, 93).

A number of autoantibodies have been described in RA patients. However, to gain relevance for a discussion of the pathogenesis of RA either a direct cell damaging effect mediated through complement or K cell activation must be made probable or a set of requirements mentioned below pertaining to immune complex mediated inflammation should be met. Very little is known about cell surface directed autoantibodies capable of inciting type II tissue injury (68) in RA, a possible example at present being IgG

antibodies to Chang cells mediating K cell cytotoxicity (137) probably due to antibody activity against a cell differentiation antigen present in the rheumatoid synovium (199). Regarding autoantibodies to soluble antigens a number of characteristics relating to the antigen and antibody would seem to be important if progressive synovitis is to be maintained: (a) The antigen must be constantly available for reaction with antibody in the joint; (b) the antibody should constantly gain access to the joint or be formed locally by the synovium; (c) antigen should be complexed to the autoantibody or at least be bound within Ig aggregates; (d) such complexes alone or in association with secondary antibodies should possess qualities which set into motion one or more inflammation provoking sequences of events such as complement activation, and lymphokine/monokine production.

With these requirements in mind only five autoantigen/antibody systems warrant interest at the present time:

A. *Rheumatoid factors*, autoantibodies to the Fc-fragment of autologous IgG, probably produced in response to immune complex bound IgG antibodies (44, 72, 89, 90, 99, 238), maybe after attachment to a population of lymphocytic cells (99, 100), are abundantly present in immune complexes of RA sera, joint fluids, phagocytic cells of the joint fluid and membrane (89, 99, 203, 239, 246). Both IgG and RF are produced by the synovium (64, 123, 131, 132, 174). IgG and IgM RF readily fix complement (20, 189) especially when present in aggregates exceeding a certain size (116, 190, 232).

B. *IgG agglutinators* are autoantibodies of IgG class against break-down products of autologous IgG or determinants of γ -chains of immune complex bound IgG (10, 59, 122, 136). They are termed pepsin, cathepsin D and neutral protease agglutinators according to their target epitopes on γ -chains revealed by treatment with these enzymes (10, 60, 121,

133, 136). Pepsin and cathepsin D agglutinator levels are abnormally high in RA sera and joint fluids (10, 122, 136). IgG split products are demonstrable in RA joint fluids (58), and agglutinators have been found in association with IgG aggregates in phagolysosomes of synovial membrane and fluid phagocytes (60). The antibodies are formed by plasma cells of the synovium (10, 60, 132) and are detectable in synovium eluates (131). The complement fixing abilities of pepsin agglutinators are negligible (122) and they may represent simply an amplified physiological clearance mechanism for split products of IgG (99). However, experimental arthritis has been produced by injections of autologous Fab₂ fragments intraarticularly into rabbits (59, 155).

C. *Immunoconglutinins* are IgG and IgM antibodies to complement components probably produced in response to immune complex bound C4 and/or C3 (120, reviewed in 108). These complement components are found in RA joint fluids (165, 246), in relation to Ig aggregates (36, 116, 246), in synovial membrane eluates (35, 131), in phagolysosomes of phagocytic cells (24, 104, 203), and in relation to Ig aggregates of the synovium (22, 23, 132). Though complement found in relation to Ig aggregates is likely to represent immune complex bound and activated products, the possibility of complement playing the role of an antigen is also very likely, since immunoconglutinin activity is released by enzymatic treatment of complexes from synovial membrane eluates as well as by immune complex dissociation of joint fluids (120, 122). Both complement components and immunoconglutinins are likely to be produced by the RA synovium (132, 166). The antibodies augment immune complex mediated inflammation through complement activation (120).

D. *Anti-collagen antibodies* may be directed to several types of native and denatured collagens (126, 182). They may belong to both the IgG

and IgM classes (8, 9, 125, 126). High titres of antibodies to denatured type I and II collagen are found in RA sera (9, 126, 183) and joint fluids (8, 37, 179). Antibodies to native type I, II and III, i.e. to epitopes on the helix structure, have also been demonstrated in high concentration in RA sera (8, 182). It has been postulated that antibodies to type II collagen are synthesized by the rheumatoid synovium (125), while other studies have indicated presence of collagen/anti-collagen complexes in RA synovial fluids (124) and in inclusions of joint fluid phagocytic cells (181). The CF potential of these antibodies is unknown, but repeated intraarticular injections of preformed collagen/anti-collagen complexes into rabbit joints result in a prolonged arthritis (180).

E. Antinuclear antibodies. Nuclear antigens are abundant in inflammatory joint fluids such as in RA (12, 115, 116, 141, 160) probably released from many types of cells in the synovial environment. Such antigens have been found in relation to Ig aggregates in the rheumatoid synovial membrane and in phagolysosomes of phagocytic lining and joint fluid cells (23), and ANA have been found concentrated in joint fluid phagocyte lysates and in synovial membrane eluates (35, 248). A local production of ANA has been postulated from comparative studies of synovial membrane eluates and joint fluids (35) as well as from ANA determinations in paired joint fluids and sera (14, 53, 69, 114, 130). Very little is known about the complement fixing abilities of ANA in RA synovial fluids, but immunofluorescence data (224) indicate modest complement fixability attributable to these antibodies, though immune complexes involving nuclear antigens and ANA may fix complement readily both in serum and in joint fluid (116, 211, 246).

Granulocyte-specific antinuclear antibodies. In the preceding sections of this review arguments have been raised for a concept which sees GS-ANA as very common participants in

immune complexes of rheumatoid sera and joint fluids (148, 216, 217, 224, 225, 229). The phlogistic properties of these complexes are likely to depend upon a number of physico-chemical and biological characteristics such as the Ig class composition, the molecular size and the complement-activating potential of the complexes (62, 99, 131, 153, 194, 232, 246). A number of these characteristics are carried by the individual specific antibodies contributing to the complexes.

Though GS-ANA are just one specificity out of several specific antibodies participating in the complexes mentioned there are reasons to believe that they have a special role in the early phase of RA. Neutrophilic granulocytes are the earliest appearing inflammatory cells arriving in the synovium and maybe the joint fluid (81, 168, 246) in the very first period of this disease. GS-ANA are commonly found in the early phase of RA (50), and thus the possibility exists that granulocyte derived nuclear antigens combining with corresponding GS-ANA constitute an early auto-immune antigen-antibody system causing ongoing inflammation by its constant formation in the joint. It is conceivable that these complexes could act as immunogens for the subsequent production of anti-immunoglobulins such as RF and IgG agglutinators, and by fixing and activating complement they could trigger immunoconglutinin production. Carefully planned parallel pathoanatomical, immunohistochemical and serological studies of very early cases of RA followed sequentially are needed to evaluate this hypothesis.

The occurrence of high titres of IgG and IgM GS-ANA and GS-ANA having a strong complement activating capacity in RA patients manifesting neutropenia might be mere epiphenomena reflecting an enhanced antibody response to granulocyte nuclear antigens released from degrading cells as a result of an increased turnover. A number of studies have postulated that the neutropenia of Felty's syndrome could be caused by autoantibodies to plasma membrane antigens of

neutrophilic granulocytes (17, 28, 39, 162). However, these studies have not duly considered the methodological problems caused by the occurrence of circulating immune complexes capable of interacting non-specifically with Fc- and C3b-receptors on neutrophils. Preliminary studies from our laboratory indicate that Fab₂-fragments of isolated serum IgG from neutropenic cases of RA are devoid of surface directed anti-neutrophilic granulocyte antibodies (150).

Recent studies rather seem to support our initial hypothesis suggesting that a quick removal of granulocytes from the circulating blood is primarily due to coating of the cells by immune complexes or even phagocytosis of such complexes of which GS-ANA are important constituents (221). Support for this concept has come from studies showing selective enrichment of granulocyte-reactive ANA (211) and GS-ANA (148, 216, 229) in circulating immune complexes of Felty patients, and the concept has been strengthened by observations indicating ongoing phagocytosis of immune complexes by neutrophils in the circulating blood of these patients (6, 78, 93). Displacement of neutrophils from midstream circulation by margination along the endothelium is fully compatible with the hypothesis as is a shortened granulocyte life span (26, 246). The interaction of immune complexes with granulocytes results in a compromised function of the cells as evidenced by a diminished chemotaxis (244), phagocytosis and bacterial killing (78, 197). This functional defect as well as a decreased neutrophil production (26, 197) in some Felty patients makes them susceptible to infection (78, 92, 93).

The pathogenetic role of complement-fixing GS-ANA is illustrated by the close relation disclosed between high titres of these antibodies in the blood and leakage of GS-ANA into the urine in neutropenic RA patients (223). Through their involvement in circulating immune complexes GS-ANA may contribute to immune complex deposition in renal glomeruli of Felty patients (223) and in the

dermoepidermal junction zone of the skin of RA patients (41, 145, 146).

Also in RA joint fluids a relation has been found between high titres of IgG GS-ANA and low neutrophil counts (224), but it is unknown whether this reflects a mere blocking effect due to higher levels of free nuclear antigens in the cell-rich fluids or if a cytopathogenic mechanism is operating comprising immune complex ingestion and removal of damaged cells. The latter possibility is supported by the observation of a diminished half-life of granulocytes in RA joint fluids (246).

The role of IgD GS-ANA is not known, but it is well established that IgD antibodies may participate in tissue immune complex deposition, and the study of Wiik & Permin (229) further indicates involvement of IgD GS-ANA in circulating immune complexes in neutropenic cases of RA. IgE GS-ANA similarly seem to be part of the circulating immune complexes so commonly found in this particular group of RA patients (148). The role of these antibodies may be that of a classical type I autoimmune inflammatory reaction adding further phlogistic potential to the complexes (147, 148).

The neutrophilic granulocyte thus seems to play a very special role in rheumatoid inflammation. It is the earliest invading inflammatory cell in the synovium in the initial phase of the disease (81, 168, 246), and in later phases it is continuously being attracted to the joint space by a number of chemotactic stimuli (24, 71, 90, 116, 165, 190, 232, 239, 246). In the joint space, and possibly on its way to this, it releases substantial amounts of lysosomal enzymes capable of causing tissue degradation (81, 85, 88, 214, 239, 246), and concomitantly it releases prostaglandins which may induce pain, vascular reactions and are likely to influence neutrophilic granulocyte function as well as lymphocyte function (74, 201, 241, 244). Experimentally, lysates of granulocyte lysosomes have been shown to produce joint inflammation, lining cell hypertrophy, fibro-

plasia and pannus formation when injected into rabbit joints (213). Neutrophilic granulocytes may function as effector cells in antibody-dependent cellular cytotoxicity if antibody coated target cells are present in the adjacent environment (67). Like many other cells granulocytes produce a connective tissue activating peptide causing increased hyaluronate production and energy metabolism, probably being one of the factors linking the acute inflammatory events with those of the ensuing proliferative mechanisms in RA (30, 81, 85). Finally, as stated in this review, granulocytes may sequester and disintegrate in the rheumatoid joint giving rise to GS-ANA production, immune complex formation and ongoing inflammation.

12. SUGGESTIONS FOR FUTURE RESEARCH ON GRANULOCYTE-SPECIFIC NUCLEAR ANTIGENS AND ANTINUCLEAR ANTIBODIES

There are important issues to study in RA patients to complete the proposed self-perpetuating inflammatory cycle involving GS-ANA. As mentioned already studies should be carried out in early cases of RA to monitor precisely the sequence of events taking place in the synovial membrane, fluid and serum as regards local cellular recruitment, local production of the various autoantibodies mentioned in section 11—especially the GS-ANA—and the possible involvement of granulocyte-specific nuclear antigens in immune complexes in the tissues, phagocytes and body fluids. Quantitative studies of local and systemic GS-ANA synthesis and catabolism in RA patients would be of interest compared with clinical manifestations. Signs of cellular immunity to granulocyte-specific nuclear antigens should be sought in order to elucidate the role of specific T lymphocyte activation for the continued production and the Ig class of GS-ANA in RA patients as compared to non-rheumatoid patients and controls having GS-ANA in serum. An increased frequency of HLA-Dw4 has been

found in adult RA patients (119) pointing towards genetic factors of importance for the development of the disease. It is not known if GS-ANA production or neutropenia correlate with specific HLA phenotypes, and such studies are warranted to shed light on genetic predispositions and prognosis. The granulocyte-specific nuclear antigens should be isolated and analysed to gain knowledge about their nature and possible pathological roles in patients as well as in experimental animals where cellular and humoral immune responses can be manipulated separately. Microbial agents capable of infecting neutrophilic granulocytes should be focused upon with special interest since such an approach might give clues as to aetiological possibilities in RA. Cross-reactivity of granulocyte nuclear antigens with constituents of possible aetiological agents might shed light on GS-ANA production as has been shown for certain autoantibodies in rheumatic fever (95, 237). Experimental arthritis induced by immunization of animals with autologous granulocyte nuclei or by intraarticular injection of autologous neutrophil chemotactic factors might help elucidate the role of granulocytes and their nuclear antigens in acute and chronic non-infectious arthritis in relation to cellular and humoral immunological and inflammatory events in the joints.

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