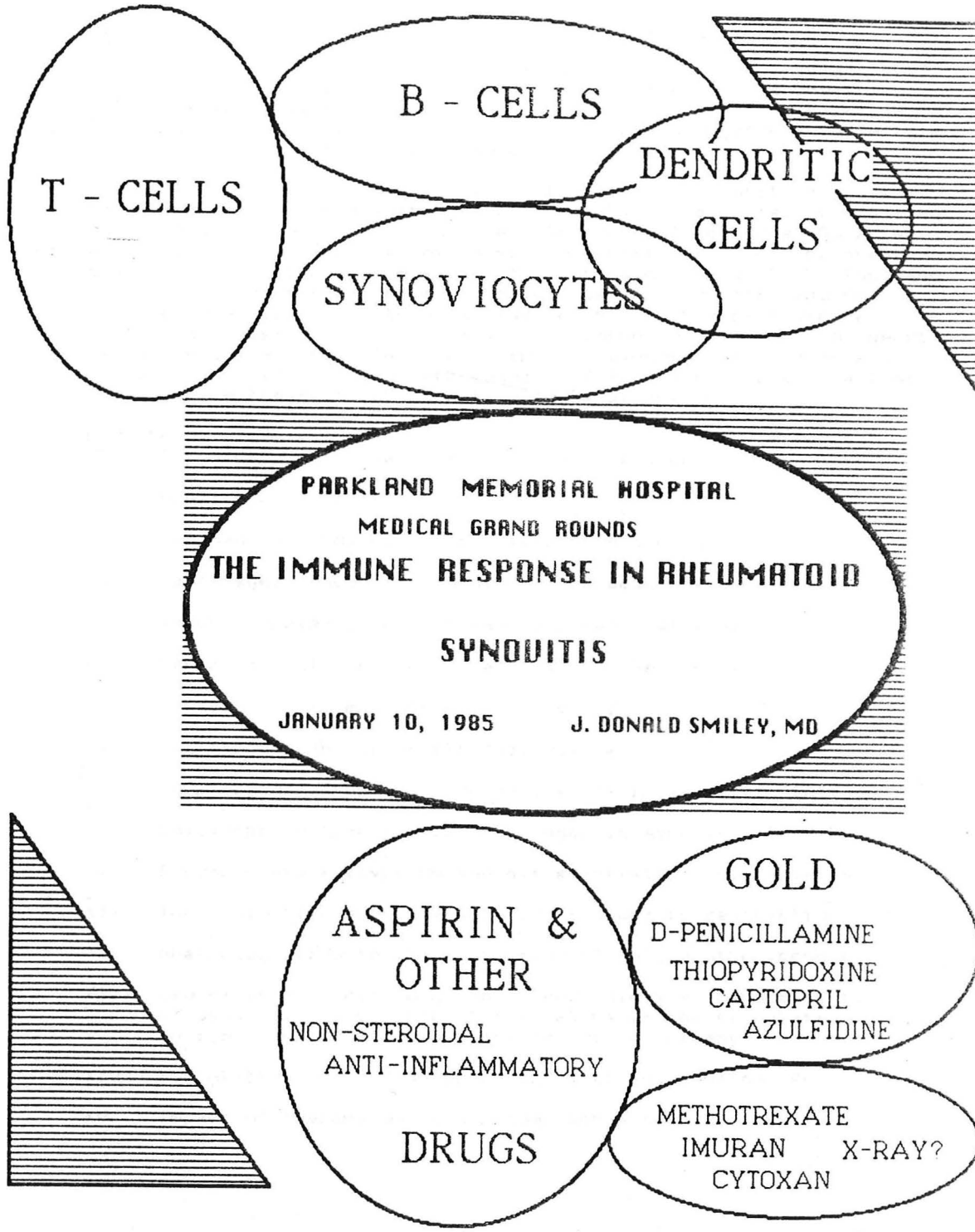

T - CELLS
B - CELLS
DENDRITIC CELLS
SYNOVIOCYTES
PARKLAND MEMORIAL HOSPITAL
MEDICAL GRAND ROUNDS
THE IMMUNE RESPONSE IN RHEUMATOID SYNOVITIS
JANUARY 10, 1985
J. DONALD SMILEY, MD
ASPIRIN & OTHER
NON-STEROIDAL ANTI-INFLAMMATORY
DRUGS
GOLD
D-PENICILLAMINE
THIOPYRIDOXINE
CAPTOPRIL
AZULFIDINE
METHOTREXATE
IMURAN
X-RAY?
CYTOXAN

THE IMMUNE RESPONSE IN RHEUMATOID SYNOVITIS

Ockham's Razor "Plurality should not be assumed without necessity." William of Ockham--13th Century philosopher.

Osler's Rule (When possible,) "all clinical signs, symptoms and laboratory abnormalities should be attributable to a a single disease process." (1)

Physicians in the past were often content to put a label on a condition such as "rheumatoid arthritis" (RA), and to tell the patient that the cause was unknown, and very little could be done (Oslerian Medicine). This is not to minimize the contributions of the great physicians of the past. However, the explosion of new technology and medical knowledge requires us to have a better understanding of disease mechanisms, and to enter the Scientific Era of Medicine. The present discussion will draw heavily on Immunology to focus on genetic background, the patient's altered immune response, and the role of possible infection in the pathogenesis of RA. The impact of all of these on the treatment of RA will also be discussed.

OUTLINE

I. Chronicity with exacerbations and remissions

II. Genetic predisposition

III. Excised RA synovium for investigative studies

IV. Cartilage, tendon and joint capsule destruction

V. Immune complexes in cartilage and synovial fluid

VI. Other immunologic factors activating the synovium

VII. Changes in T-lymphocytes in synovitis

VIII. Macrophages and dendritic (veiled) cells

IX. Lymphocyte/macrophage-induced changes in synovial fluid

X. Polymorphonuclear leukocyte changes in synovial fluid

XI. B-Lymphocyte activation and differentiation in synovitis

XII. Immunoglobulin subclass and electrophoretic restriction

XII. Analogous oligoclonal restriction of IgG in other conditions

XIII. Use of isoelectric point (pI), and idiotype characteristics of synovial IgG to predict the nature of the stimulating antigen, and to develop possible future therapy

XIV. Speculations regarding potential antigens causing RA

XV. Impact of therapy on immune components of RA synovitis

CHRONICITY, EXACERBATIONS AND REMISSIONS

Rheumatoid arthritis (RA) is a common, clinically highly variable condition characterized by exacerbations and remissions, and a destructive polyarthritis (2,3). Over 80 % of patients develop RA between the ages of 35 and 50 with women involved twice as often as men. The first and dominant feature of the illness is the synovitis, which some investigators view as generated predominantly by inflammatory mediators (lymphokines) released by T-lymphocytes (4,5). However, in addition to arthritis, almost all patients have extraarticular signs and symptoms of systemic disease with weight loss, mild fever, morning stiffness of muscles, and anemia most frequent. There is an increase in mortality 2.5-fold over matched control populations with RA listed as the cause, or a contributory cause of death in 38% of patients (6).

GENETIC PREDISPOSITION

The reasons for the predilection of RA for middle-aged or older persons, and for women over men is unknown. However, beginning with the classic family study of Ziff, et al. (7) in 1958, a genetic susceptibility for RA and rheumatoid factor production has been known. Stastny solidified this genetic association when he discovered the increased frequency of HLA-D4 (54% compared to 16% of non-RA controls) (8), and of HLA-DR4 (70% compared to 28% of non-RA controls) (9) in white patients with RA. When families with multiple members with RA are typed, this association with HLA-DR4 rises to 90% (10)! These genes are much less frequent, although still increased, in RA patients who are Black, Latin American or of American Indian origin. This racial variation suggests that other genetically determined factors, in addition to HLA-D4 and HLA-DR4, may contribute to disease susceptability in RA. Studies of other loci on the sixth chromosome in the histocompatibility region have shown HLA-Cw3 (11,12), HLA-A1 (12) and certain combinations: (See Table I) HLA-A1/DR4, HLA-A1/MT2, HLA-Bw40/Cw3, HLA-Cw3/DR4, HLA-Bw40/DR4, and HLA-DR4/MT2 to contribute substantial additional risk for the development of RA (12). The increased association of rheumatoid factor with HLA-DR4 or HLA-D4 has been debated (10,13,14). It is probably increased. However, it should be noted that the capability to make rheumatoid factor exists in blood lymphocytes of most normal persons (15). The above findings support multigenic factors in disease susceptability in RA. However, the gene products on the cell surface do not cause RA. All of the gene types mentioned above are present in many times greater frequency in normal persons than that of RA which occurs in only 1-1.5% of the population (4).

FIGURE 1.

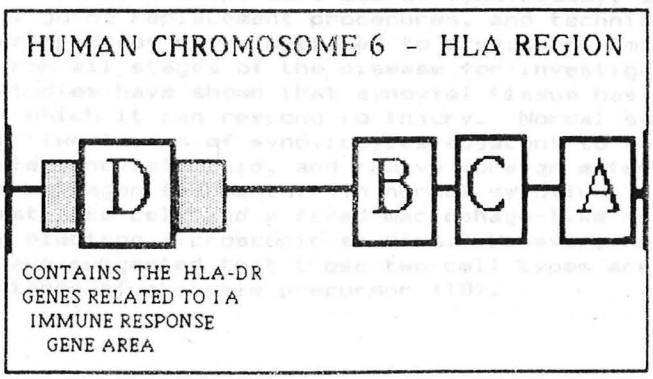


TABLE I. GENETIC COMBINATIONS PREDISPOSING PATIENTS TO RHEUMATOID ARTHRITIS

(Modified from Karsh, et al, 1983, Ref. 12)

SPECIFICITY	CONTROLS	RA PATIENTS	$P(X^2)$
HLA-DR 4	35 %	66 %	<0.001
HLA-CW 3	18	33	<0.005
HLA-A 1	25	44	<0.025
HLA-DRW 6	28	11	<0.01
HLA-DR 4/ MT 2	3	23	<0.001
HLA-CW 3/ DR 4	8	31	<0.001
HLA-A 1/ DR 4	5	25	<0.001
HLA-A 1/ MT 2	11	37	<0.001
HLA-BW 40/ DR 4	3	18	<0.002
HLA-BW 40/ CW 3	3	17	<0.003

Further genetic considerations in RA should be made. In addition to the predilection, particularly for milder disease, for women (4), there is no increase in a known genetic allotype with the extraarticular manifestations of RA, such as Felty's or Sjogren's syndrome (12), and the frequency of HLA-DR-4, HLA-A-1, and HLA-MT2 may be similar in patients with and without rheumatoid factor (12).

TABLE II. FURTHER GENETIC CONSIDERATIONS IN RHEUMATOID ARTHRITIS

1. MORE WOMEN THAN MEN HAVE RA (2:1 F:M)
2. NO PARTICULAR GENETIC ALLOTYPE HAS BEEN ASSOCIATED WITH THE EXTRAARTICULAR MANIFESTATIONS, SUCH AS FELTY'S OR SJOGREN'S
3. FREQUENCY OF DR-4, A-1, AND MT-2 SIMILAR IN RHEUMATOID FACTOR POS & NEG PATIENTS
4. LESS HLA-DR-4 IN NON-CAUCASIAN PTS WITH RA

EXCISED RHEUMATOID SYNOVIUM FOR INVESTIGATIVE STUDIES

The documented usefulness of synovectomy of the wrist (16) has not been matched by similar benefit in long-term follow-up of the consequences of synovectomy of weight-bearing joints, such as the knee (17). However, the continued sporatic use of synovectomy, the frequency of total joint replacement procedures, and technical improvements in arthroscopy have continued to provide rheumatoid synovial tissue from all stages of the disease for investigative studies. These studies have shown that synovial tissue has a limited number of ways in which it can respond to injury. Normal synovium is composed of one or two layers of synoviocytes adjacent to the joint space which secrete synovial fluid, and remove foreign material or worn cartilage or collagen fragments. In normal synovium, two cell types, a fibroblast-like cell and a fixed macrophage-like cell have been described by electron microscopic studies. However, recent organ culture studies have suggested that these two cell types are merely differentiation stages of a single precursor (18).

With the onset of inflammation, radical changes in synovial membrane stucture and histology occur. These are non-specific, with RA producing more intense changes than other seronegative arthropathies (19). The recent availability of monoclonal reagents for identifying HLA-DR (Ia) and monocyte-macrophage antigens has provided a better functional definition of synoviocytes, and allowed division into three types (I, II, and III) (20,21, 23). Synoviocytes near the joint surface (Type I) have surface receptor proteins like activated macrophages (macrophage+/Ia+). An intermediate cell (Type II) located in the deeper layer of the synovium around blood vessels and in areas of lymphoid cells contains no macrophage surface protein, but is Ia+, compatible with activation by the inflammatory process. Beneath the Type I cells, there is a broad layer of Type III cells which have characteristics of fibroblasts (24) (macrophage-neg/Ia-neg). Histopathological studies using monoclonal reagents with the peroxidase technique on frozen sections of RA synovium have shown synoviocytes to be surrounded by legions of T-lymphocytes which are predominantly Ia+ suggesting an activated state, and intermixed with mononuclear cells having surface proteins characteristic of natural killer cells (HNK 1+) (22).

When synoviocytes are examined in tissue culture (25,26), an adherent population which spreads to form a stellate structure and which secretes collagenase and prostaglandin E2 (PGE2) can be isolated. This stellate cell lacks a surface receptor for IgG-Fc and does not secrete lysozyme, suggesting that it is not a macrophage-type cell. Its activation can be induced by a soluble factor secreted by blood mononuclear cells and/or by additional PGE2 (26,60).

In addition to the intense proliferation of synviocytes, and infiltration of lymphoid cells into rheumatoid synovium, significant blood vessel changes, not unlike the capillary basement membrane changes seen in diabetes mellitus, occur. There is thickening of the capillary basement membrane (27), and increased deposition of the high molecular weight structural glycoprotein, laminin (28).

When rheumatoid synovitis is acute, the middle layer of the synovium becomes infiltrated with reticulin as defined by silver-staining techniques (29). This reticulin is composed of the cement protein, fibronectin, of a non-collagenous reticulin component described by Pras and Glynn, and of Type III collagen fibers (29). Later, in the more chronic phase of rheumatoid synovitis, the reticulin fibers are replaced with a dense collagen network. In addition to its contribution to reticulin, fibronectin is found throughout the synovium where it is produced locally by the Type B fibroblast-like synoviocytes (30,31). Fibronectin, present in small amounts in serum, is also found in large amounts in RA synovial fluid (32,33). Fibronectin cements cell to cell, and cell to adjacent collagen or reticulin fibers. The large amount of fibronectin in synovial tissue explains the the difficulty encountered when rheumatoid synovial tissues are teased in an effort to disperse the infiltrating cells into isolated cell suspensions. Fibronectin traps monocyte/macrophage-like cells, B and T-lymphocytes, and dendritic cells, all of which originate elsewhere, in a sessile form in the synovium.

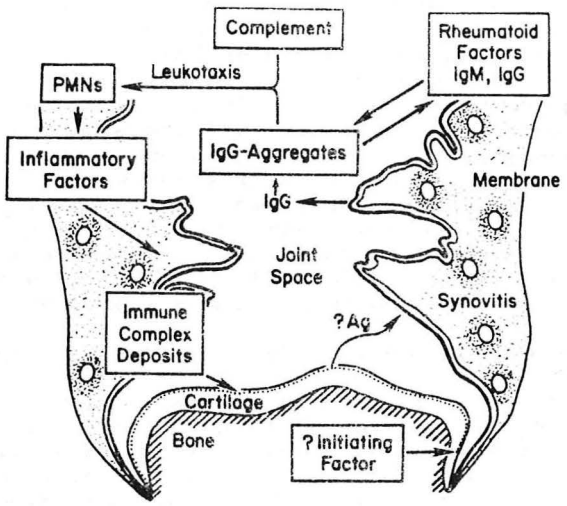
CARTILAGE, TENDON, AND JOINT CAPSULE DESTRUCTION BY PANNUS

Although pitting of the joint cartilage may result from enzymes released by polymorphonuclear leukocytes in joint fluid, studies of thousands of rheumatoid joints have convinced most investigators

(34,35,37) that the action of highly-activated ("aggressive"), macrophage-like cells at the cartilage/ pannus interface cause most of the erosive changes of RA. The pre-existing immune complex-like deposits (see below) at the very surface of RA cartilage and other joint connective tissues can be visualized as the stimulus for overgrowth of pannus, and these complexes disappear from the cartilage under the pannus layer. Cartilage erosions, like later bony erosions, are filled with outgrowths of small blood vessels surrounded by aggressive synoviocytes, which alter cartilage metabolism, and lead to collagen and proteoglycan destruction in hyaline cartilage (36). Some of these synoviocytes become multinucleated ("chondroclasts"), and assume the form of osteoclasts (38). It is also probable that the inflammatory mediators from the adjacent synovium activate other osteoclasts (39) and the cartilage chondrocytes (40), also leading to digestion of collagen and proteoglycans in bone and cartilage. The microvascular changes within the RA synovium enhance the transfer of proteins such as fibrinogen and complement components into the joint, while at the same time, because of the marked increase in the number of cell layers between blood vessels and the joint space, significantly decrease synovial permeability for small molecules such as glucose (41).

Collagenase secretion depends upon signals received which activate the fibroblastic synoviocyte fraction. Aggregated IgG stimulates collagenase secretion 25-fold, for example, in synoviocytes and chondrocytes (42,43), possibly by stimulation of a "mononuclear cell factor" homologous with interleukin-1 (43). This is an additional observation which suggests that the immune complex-like deposits in the RA cartilage surface may be the stimulus for pannus overgrowth and cartilage destruction. In addition to collagenase, plasmin and other proteases are released which have the potential to cause joint damage (45). However, the sharp localization of damage to selected areas of the joint results from the large amount of protease inhibitors, such as alpha2-macroglobulin, present in RA synovial fluid (44,46) and in serum.

FIGURE 2. SCHEMATIC REPRESENTATION OF RHEUMATOID SYNOVITIS



IMMUNE COMPLEXES IN CARTILAGE AND IN SYNOVIAL FLUID IN RA

Figure 2 above diagramatically illustrates the thin layer of immune-complex-like material present near the cartilage surface in all patients with RA (47,48). This material forms an amorphous aggregate in the electron microscope, and contains IgG, IgM, C1q, and C3 (47). Soluble immune complexes are present in rheumatoid synovial fluid (49,50), and in phagocytic inclusions from polymorphonuclear leukocytes from joint fluid and blood (51,52,53). IgM-and IgG-rheumatoid factors form part of these immune complexes. However, studies of early RA has shown immune complex constituents to be synthesized within the synovium before detectable levels of rheumatoid factor (50).

As suggested above in explaining the pannus overgrowth of cartilage, followed by erosions of cartilage and underlying bone, the presence of the sub-surface immune complexes must be seen as a central feature in the pathogenesis of RA. The presence of similar, although much less dense, deposits in an occasional patient with "erosive osteoarthritis", and the absence of such deposits in other types of inflammatory arthritis is of significant interest, and suggests a closer link between erosive osteoarthritis and RA than was previously suspected. In RA, gradual release of trapped putative antigen from this cartilage site may provide the chronic stimulus to maintain RA synovial proliferation for many years. Mechanical agitation of cartilage containing such complexes explains the frequent flare-up of RA which follows vigorous exercise; and the lack of movement, the total disappearence of arthritis on only one side of the body after hemiparesis. This hypothesis is probably best supported by the observation that removal of all of the cartilage, including that on the back of the patella, from a joint at the time of total knee replacement results in complete disappearence of synovitis from the joint, while leaving the patellar cartilage intact usually results in a prompt return of active synovitis (3).

Recent studies have shown that the normal complement system can solubilize and inactivate immune complexes such as those in the RA cartilage surface, in synovial fluid and in the serum of RA patients. Rheumatoid synovial fluid shows a marked decrease in this ability when compared to synovial fluids from patients with osteoarthritis (54). There was a decrease in immune complex solubilizing ability in 65% of rheumatoid synovial fluids with this decrease correlating best with a decrease in function of the alternative complement pathway, and the level of immune complexes already present in the synovial fluid tested. It was concluded that the results confirmed a possible role of the synovial complement system in modulating the size and thus the diffusion and clearance of locally generated immune complexes (54). Other studies have also shown prevention of immune precipitation by purified classical pathway complement components (55). What therapeutic role perfusion of the joint with complement components might have in inducing a remission of RA is unknown at this time.

OTHER IMMUNOLOGIC FACTORS ACTIVATING THE RHEUMATOID SYNOVIUM

When RA synovial tissues are propagated in tissue culture, a synovial fibroblast cell line often may be obtained. When such cell lines are tested with RA serum known to contain anti-collagen antibodies, no detectable binding is found, and no IgG of any type can be detected on the surface of these synoviocytes with a sensitive method (56). This would be against a fibroblast-associated antigen causing the immune response in the synovium in RA. However, analysis

of anti-lymphocyte antibodies in the serum of patients with RA has shown autoantibodies reactive with the Ia antigen which is present on Type I and Type II synoviocytes, macrophages, dendritic cells, activated T-lymphocytes and some vascular endothelial cells in the synovium, and, thus might enhance inflammation involving these cell types. The titer of this anti-Ia antibody shows a positive correlation with the severity of arthritis in the RA patient donating the serum (57). These authors (57) speculated that these anti-Ia antibodies could play a role in the regulation of immune reactions in RA synovitis.

The complement systems also play an active role in RA with decreased C3 and CH50 levels in the synovial fluid of RF-positive patients with active disease. This is significant with respect to the synovial lining layer cells since they contain receptors for both C3b and IgG-Fc (58) and are further activated by C3b, or by complexes containing C3b and IgG-Fc. In addition, increased levels of C3c and C3d (breakdown products of C3b) are found in synovial fluid and increased levels of C3d are present in the serum of patients with active RA (59).

CHANGES IN T-LYMPHOCYTES IN RHEUMATOID SYNOVITIS

Mouse monoclonal antibodies which are specific for the various cell surface proteins characteristic of different phases of lymphocyte differentiation (61) are now commercially available. The use of these reagents in immunohistological techniques involving peroxidase staining of frozen sections of tissues and examination by both light and electron microscopy have enlarged our understanding of the role of different types of lymphoid cells in rheumatoid synovitis (62,63,64). More than anything else, these studies have shown the variability from patient to patient in the character of the rheumatoid synovial lymphoid infiltrate. Even within the synovial tissue of a single patient or from a single joint, there is variability in cell types present (62).

TABLE III. RA SYNOVIAL IMMUNOHISTOLOGICAL FEATURES

HYPERCELLULAR ANERGIC	HYPOCELLULAR NONANERGIC
↑ T-CELLS (T3)	↓ T-CELLS (T3)
↑ HELPER (Leu 3A,T4)	↑ FIBRIN
↑ HLA-DR+	
PERIPHERAL BLOOD	
↑ SUPPRESSOR (Leu 2, T8)	Nl. or ↑ HELPER T-CELLS
↑ HLA-DR+	Nl. SUPPRESSOR T-CELLS
↑ Ig-SECRETION	Nl. Ig-SECRETION

(MODIFIED FROM DECKER, ET AL. ANN INT MED 101: 810, 1984)(Ref. 4)

However, from these laborious studies, certain generalizations may be drawn. Those patients having hypercellular synovia are often clinically indistinguishable from those with more fibrinous, hypocellular synovia in terms of the severity of their arthritis. However, a number of studies (5,65,66,67) have recently suggested that these two groups of patients differ substantially in terms of the immune function of the T-cells in the synovium and circulating in the blood. These differences have been compared to anergic (lepromatous, lepromin neg.) and non-anergic (lepromin positive) leprosy (4).

An average of 5 biopsies with an arthroscope from random areas of synovium were necessary to establish the hyper-versus-hypocellular status of the RA patient (4,5). The RA synovial and peripheral blood immunohistological features of these two groups of RA patients are summarized in Table III. Of equal significance is the difference in response of these two groups of patients to leukopheresis. The hypercellular (anergic) group shows improvement, while the hypocellular (non-anergic) group usually does not respond with significant clinical improvement (4).

MACROPHAGES AND DENDRITIC (VEILED) CELLS IN RHEUMATOID SYNOVITIS

Two of the types of accessory cells capable of processing and presenting antigen to T-lymphocytes are in RA synovium. The monocyte/macrophage cell has long been recognized as a significant component of the cellular infiltrate (62). Recently the dendritic ("veiled") cell, another accessory cell which differs from macrophages in appearence, has been described (68). Dendritic cells have a high density of membrane HLA-DR+ (Ia) proteins, and are similar to cells in the paracortex of lymph nodes or the Langerhans cells in skin. They have extending and retracting cytoplasmic processes, and have a high cytoplasm to nuclear ratio characteristic of "basket or veiled" cells (69). When isolated from RA synovial fluid, dendritic cells convey a 100-fold stimulation of 3H-Thymidine incorporation when added to limiting numbers of normal peripheral blood lymphocytes, with or without added Con A, a lectin mitogen from jackbeans (69). Macrophages, when stimulated by Con A or IgG-Fc fragments also release a soluble factor which stimulates Type III synoviocytes to release collagenase (70) and arachidonate cyclo-oxygenase products (60,71).

LYMPHOCYTE-INDUCED CHANGES IN RA SYNOVIAL FLUID

RA synovial membrane secretes and is the source of the cell population within synovial fluid. Most of the leukocytes in synovial fluid are polymorphonuclear (PMN) cells, but 10 to 30% are lymphocytes and monocyte/macrophage-like cells. The lymphocytes are similar in mix to that of the peripheral blood. However, the synovial fluid mononuclear cells differ in function from those of the blood showing less response to mitogens like Con A and PHA, and more response to bacterial/fungal antigens like killed E. coli or Candida (72). These same cells, again unlike blood mononuclear cells, failed to form antibodies when stimulated by poke weed mitogen (PWM), but restored macrophage-depleted blood mononuclear cells ability to respond to PWM and form antibody (73). Synovial fluid monocyte/macrophage cells release an interleukin-1-like factor (74) which in turn stimulates T-lymphocytes (75,76) in synovial fluid to release interleukin-2 (78,79,80). Other T-cell lymphokines such as interferon (82), have also been found in RA synovial fluid. A paradoxical suppression of natural killer (NK) activity has been noted with RA synovial fluid adherent cells (monocyte/macrophages) when compared to the stimulation of NK cell activity noted when blood monocyte/macrophages from the same patient were studied (81).

Thus, the joint fluid non-PMN cell population is similar to the activated immune cells responding in the adjacent synovium with T-lymphcytes of all types, B-lymphocytes, monocytes/macrophages, dendritic cells and natural killer cells all present. However, the frequency of T4+ lymphocytes is lower than that of the synovium or blood. Also, the number of dividing lymphocytes in RA synovial fluid ranged from 2.2 to 7.2% compared to less than 1% in normal blood

lymphocytes (83). The non-dividing lymphocytes in synovial fluid had greater amounts of RNA per cell suggesting an activated cell with much more protein synthesis (83).

POLYMORPHONUCLEAR LEUKOCYTE CHANGES IN RA SYNOVIAL FLUID

PMNs, which make up 60 to 90% of the white cells in RA synovial fluid, enter the joint space because of the release of chemotactic factors such as C5a which is released with complement activation by immune complexes in the joint. However, other factors in RA synovial fluid, such as immune complexes themselves also "activate" PMNs as measured by increased chemiluminescence (84), and other metabolic and secretory activities (85). The immune complexes taken up by PMNs probably originate from Ig and complement produced in the adjacent synovium, but recent qualitative immunoelectronmicroscopic analysis, using the peroxidase-antiperoxidase method, showed membranous, vesicular, and granular unstained materials in the same vacuoles containing stained IgG, IgM, and C3. Only occasional vacuoles within PMNs contained IgA and fibrinogen (86). These studies showing other components in addition to IgG, IgM and C3, emphasize the oversimplification inherent in the contention that rheumatoid factor containing complexes are the only cause of inflammation in RA, although RF may well augment the phlogistic properties of other immune complexes.

In addition to C5a, products resulting from the lipoxygenation of arachidonic acid, such as 5-HETE and leukotriene B4, are also released into synovial fluid and are chemotactic for PMNs and eosinophils (87,88,89,90). These factors have been shown to be released by the adjacent synovium (87), and to enhance the expression of C3b receptors and increase intracellular c-GMP in PMNs. The lipid peroxidation of membrane arachidonic acid is enhanced by iron (88) which tends to be low, and by copper (90) which tends to be high in RA. Superoxide dismutase, glutathione peroxidase, and catalase which are the body's protection against the proliferation of damage from the intense activation of PMNs, have all been measured in synovial fluid (91). Superoxide dismutase is similar in concentration to that in the blood, glutathione peroxidase is only slightly increased, but catalase which also destroys peroxide, is markedly increased in RA synovial fluid (91). Were it not for the superoxide dismutase and catalase in RA synovial fluid, the intensely activated PMNs there would release enough oxygen intermediates to aggregate normal IgG, and to impair T-cell differentiation and metabolism in the adjacent synovium (92).

B-LYMPHOCYTE ACTIVATION AND DIFFERENTIATION IN RHEUMATOID SYNOVITIS

In RA patients whose disease is limited to arthritis, increased immunoglobulin synthesis by B-lymphocytes can be demonstrated in the synovial fluid, but usually not in the B-lymphocytes of the blood (93,94). On the other hand, those patients with extraarticular features such as rheumatoid nodules, vasculitis, or splenomegally, have a large increase in blood B-lymphocytes which secrete immunoglobulin to levels about 6-fold above that of a control non-RA population (93). A B-cell differentiation factor has been found in synovial fluid from patients with active RA which can substitute for T-cells in the presence of poke weed mitogen (PWM) to cause polyclonal activation of B-cells to stimulate immunoglobulin synthesis (94). This factor is present *in vivo* and activates B-cells in the synovial fluid to produce IgG, but very little IgM or IgA (95). However, synovial fluid B-lymphocytes, in contrast to blood B-cells from the

same patient, are poorly responsive to PWM (95).

One of the best models for study of the sequence of response to antigen by the synovium was developed by Dr. Hugo Jasin in 1969 (97). When a rabbit, previously immunized with bovine serum albumin (BSA) with a high serum level of anti-BSA, was given a small amount of BSA into the knee joint, an Arthus reaction occurred followed by a chronic arthritis which resembled RA. This arthritis would last up to 100 days, and during the more acute phase, would show synovial proliferation which could be excised and studied for local anti-BSA production, along with simultaneous study of anti-BSA production by the same rabbit's spleen, lymph nodes and peripheral blood lymphocytes. The surprising results are shown in Table 4. These indicate that at least 39% of the IgG being made in the joint is specific anti-BSA antibody. The remaining immunoglobulin could be the result of non-specific polyclonal B-cell activation, or more likely a humoral response to concealed antigenic determinants exposed by macrophage degradation of the BSA molecule, or related to Idiotype:anti-Idiotype regulation of the immune response (to be discussed below) to BSA. At a later time, when the joint tissues continued to make similar amounts of anti-BSA, the spleen, lymph nodes, and peripheral blood lymphocytes were found to be producing almost no anti-BSA, having now responded to other antigenic stimuli experienced by the rabbit. This persistence of local antibody synthesis in the joint which had been injected occurred because of trapping of BSA in the cartilage surface with slow release to keep up the synovial lymphoid stimulation (97). The same mechanism is postulated to occur as a result of the immune complex-like deposit which is found in the cartilage of all patients with RA (48).

TABLE IV. Ig AND SPECIFIC ANTIBODY MADE BY SYNOVIUM, SPLEEN, & PBL

(MODIFIED FROM JASIN & ZIFF, J IMMUNOL 102 355, 1969)(Ref. 97)

Tissue	Ig as % soluble protein	Anti-BSA as % total Ig
Normal Synovium	3	5
Immune Synovium*	6	39
Peripheral Blood WBC*	8	28
Immune Spleen *	74	67

* Anamnestic rabbit given BSA into knee joint

B-lymphoblasts and plasmablasts can be localized around blood vessels and in close contact with monocyte/macrophage-like cells and fibroblasts in the transitional area of the RA synovium (96). In the electron microscope, using the peroxidase staining method, 58.4% of Ig-synthesizing cells were producing IgG, and 9.2%, IgM. Previous studies of excised synovial tissues (98,99) have shown that RA synovia produce as much immunoglobulin in tissue culture as a similar weight of spleen or lymph node. Most of this synovial immunoglobulin is IgG which differs from the IgG circulating in the serum. For example, repeated immunization of the RA patient with tetanus toxoid prior to surgical synovectomy fails to stimulate the lymphoid infiltrate in the synovium to secrete anti-tetanus antibody, although the patient's circulating B-lymphocytes show an anamnestic response to tetanus (99).

In addition, RA synovial tissue has been shown to synthesize increased levels of the IgG-3 subclass both by fluorescent staining of synovial plasma cells with subclass specific antisera (100), and by fractionation of radioactively-labeled IgG from the culture supernatant on staphylococcal Protein A (101). Since an average of only 5 to 9% of this synovial IgG represents IgG-rheumatoid factor (anti-IgG) (101,123), most synovial IgG must have some other antigenic specificity.

Finally, when the purified IgG from RA synovial cultures is examined for its electrophoretic mobility using isoelectric focusing (IEF), 50% of RA synovia are found to produce a highly restricted (oligoclonal) IgG (102). This oligoclonal restriction of IgG was found to spill over into the serum of 17% of patients with active RA in sufficient amount to be visible above the normal IgG background (102). The degree of this restriction is shown by Fig. 3 and Fig. 4 in which RA synovial IgG and RA serum IgG are compared to normal human serum IgG using IEF in agarose gel.

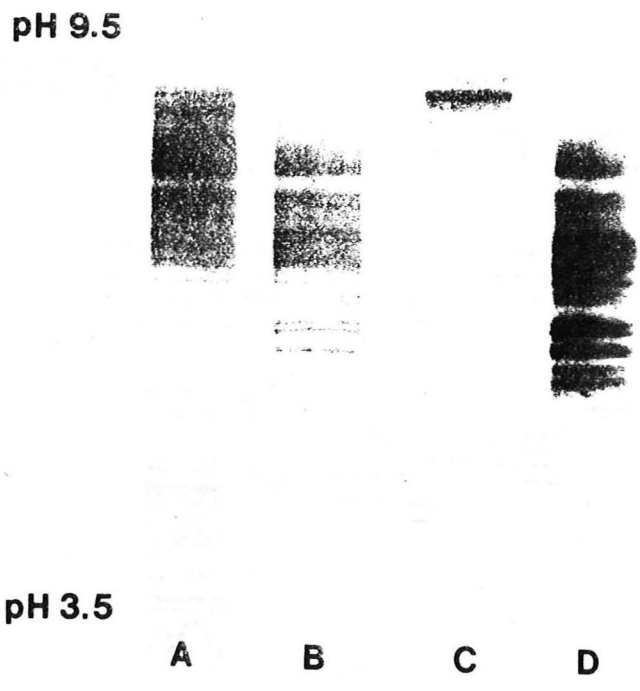


FIGURE 3. Comparison of IEF banding of the IgG from matched serum of M.J. stained with Coomassie Blue (A), with a radiofluorograph of 14-C-IgG from the synovial culture of M.J., (C), and with that of IgG from pooled normal serum stained with Coomassie Blue (B), and seen by radiofluorography after being labeled with 125-Iodine (D).

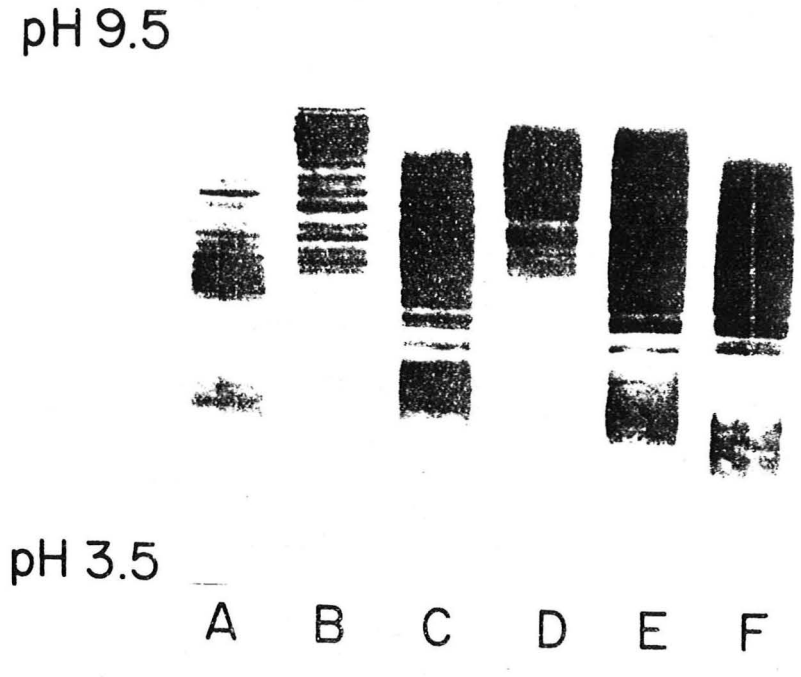


FIGURE 4. Radiofluorograph of IEF patterns of 14-C-labeled IgG from 4 RA synovial culture supernatants (A-D) showing electrophoretic restriction compared to a 14-C-labeled culture supernatant IgG showing no enhanced IEF banding (E), and to 125-I-labeled, normal human IgG (F) (Ref. 102).

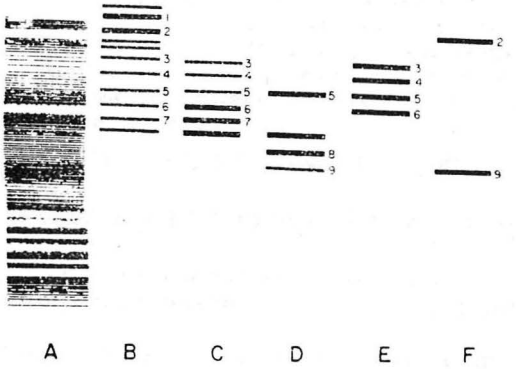


FIGURE 5. Schematic presentations of the various patterns of enhanced IEF bands noted in RA synovial and serum IgG. Panel A = Coomassie blue stained normal human IgG pool, Panels B,C,D = Synovial IgG from 3 RA patients, Panels E,F = Serum IgG from 2 RA patients. Panels B-D are from radiofluorographs of IEF separations in agarose gel (Ref. 102)

In addition, when RA synovial IgG is fractionated with chromatofocusing, a technique which separates IgG according to isoelectric point (pI), it was found that most of the patients produced synovial IgG with an increased isoelectric point (pI). This is illustrated in Figure 6 below.

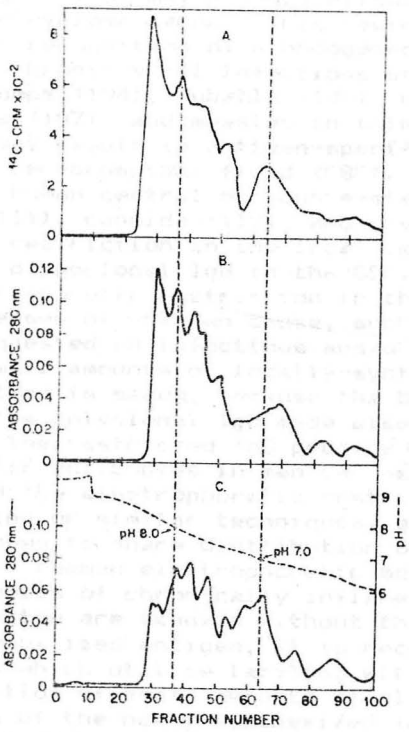


FIGURE 6. Comparison of cathodal shift in isoelectric point (pI) on a chromatofocusing column of synovial culture supernatant IgG of M.J., (A), and of the serum IgG of M.J. (B), with IgG from a pool of normal sera (C). Each fraction was done sequentially on the same column after washing with 1.0 M NaCl between samples. The vertical lines indicate pH 8 and pH 7 (Ref. 102).

TABLE V. CHARACTERISTICS OF RA SYNOVIAL IgG

1. 8-26% of soluble protein made by synovium (Ref. 98)
2. Contains different antibodies from serum, even after exogenous immunization of patient (Ref. 99)
3. 5-9% of synovial IgG is rheumatoid factor (Ref. 102)
4. An average of 22-41% is IgG-3 (serum=7%) (Ref. 101)
5. More alkaline pI. Oligoclonal in 50% patients (Ref. 102)

ANALOGOUS OLIGOCLONAL RESTRICTION OF ANTIBODIES IN OTHER DISEASES

Repeated or chronic immunization of animals or patients often induces an electrophoretically-restricted population of specific antibodies. For example, rabbits given streptococcal group specific carbohydrate antigen (103) may produce oligoclonal IgG which resembles the IgG present in myeloma serum. This restricted humoral immune response indicates recognition of a homogeneous antigenic determinant. In man, acute and chronic viral infections of the central nervous system, such as mumps (104), rubella (105), herpes simplex (106), Epstein-Barr virus (107), and measles in sclerosing panencephalitis (SSPE) (108,109) may result in antigen-specific, oligoclonal IgG detectable in the cerebrospinal fluid (CSF). Bacterial and fungal infections of the human central nervous system, such as tuberculosis (110), syphilis (111), candida (112), and cryptococcus neoformans (113) also induce restriction in the local immune response leading to antigen-specific, oligoclonal IgG in the CSF. In addition, the presence of electrophoretic restriction in the IgG in the CSF of patients with diseases of unknown cause, such as multiple sclerosis (114,115), has suggested an infectious and/or other immune etiology.

Detection of small amounts of locally-synthesized, oligoclonal IgG is easier in CSF than in serum, because the blood-brain barrier excludes most of the polyclonal IgG made elsewhere which might otherwise obscure the restricted IgG produced in the central nervous system. If specific antibodies in man can be isolated by affinity chomatography, and the electrophoretic restriction examined by isoelectric focusing or similar techniques, a high percentage of patients can be shown to share distribution of antibodies to some determinants with a common electrophoretic mobility (116). However, when humoral responses of chronically inflamed tissues outside of the central nervous system are studied without the benefit of antibody selection with immobilized antigen, it is necessary to use tissue culture techniques which utilize labeling with radioactive amino acids to show the production of small amounts of oligoclonal IgG. This avoids the masking of the newly-synthesized IgG by serum IgG contamination. Using these culture techniques, the IgG produced by RA synovial fragments in tissue culture has been examined by isoelectric focusing (IEF) and by chromatofocusing for restriction in electrophoretic mobility. The results show that RA synovia often produce IgG which is similar to the oligoclonal IgG found in the CSF from patients with defined infections of the central nervous system (102).

TABLE VI. POSSIBLE SIGNIFICANCE OF ELECTROPHORETIC RESTRICTION OF RHEUMATOID SYNOVIAL IgG

1. SPECIFIC ANTIBODIES TO CNS INFECTIONS IN CSF (VIRAL, BACTERIAL, FUNGAL) ALSO OLIGOCLONAL
2. BY ANALOGY, SYNOVIAL IgG RESTRICTION ALSO SUGGESTS A LOCAL, CHRONIC ANTIGENIC STIMULUS
3. ISOLATION OF RA SYNOVIAL OLIGOCLONAL ANTIBODIES IN PURE FORM PERMITS GENERATION OF RABBIT AND MOUSE ANTI-IDIOTYPIC REAGENTS TO TEST FOR A COMMON AGENT IN RA. ALSO TO OBTAIN MORE SERUM ANTIBODY FOR VIRAL AND BACTERIAL SCREENING FOR AN ETIOLOGIC AGENT

USE OF ISOELECTRIC POINT (pI), AND IDIOTYPE CHARACTERISTICS OF SYNOVIAL IgG TO PREDICT THE NATURE OF THE STIMULATING ANTIGEN AND TO DEVELOP POSSIBLE FUTURE THERAPY FOR RA

Similar to the synovial IgG shown in Fig. 6, the IgG from 82% of the RA synovial supernatants showed an increase in cathodal migrating IgG (102). The IgG from the matched sera of the same patients showed this cathodal shift in 3 of the 11 samples tested. The cathodal shift has been observed to persist for over two years in the serum of one RA patient, and is present in the synovial IgG obtained from patients with widely differing duration of RA. To rule out the possibility that IgG with a more basic pI was selected by the *in vitro* culture technique and/or the isolation on the anti-IgG immunoadsorbent, the peripheral blood lymphocytes from 9 normal persons were stimulated with pokeweed mitogen, a non-specific B-lymphocyte activator. The resulting IgG which was synthesized *in vitro* was isolated using the same methods as applied to the RA synovial culture supernatants. None of these control peripheral blood lymphocyte cultures produced IgG with a cathodal shift in pI, and 8 out of 9 showed a more acidic distribution of IgG than that observed in normal sera.

Vandvik (106,115) also showed a relative increase in cathodal IgG in most of the abnormal CSF samples from multiple sclerosis, and in SSPE and other infections of the central nervous system. He did not find a similar increase in cathodal shift in the IgG from the matched sera from these patients. This suggests that the frequently observed cathodal shift in the average pI of synovial culture IgG results from a local immune stimulus in the joint, which is not usually present in other non-articular lymphoid tissues.

Previous studies have suggested that antigenic determinants which are acidic tend to elicit antibodies of higher pI. Sela and Mozes (117) showed that acidic amino acid copolymers elicit a basic antibody response, while more basic amino acid copolymers elicit acidic antibody. Thus, the cathodal shift in pI of synovial culture IgG might reflect a relatively acidic stimulating antigen(s). In the experimental immune arthritis produced with BSA in mice, when the BSA is first methylated making it very alkaline, there is greater localization and persistence of antigen in the joint, and the arthritis is more chronic than when relatively acidic BSA is used as the immunizing antigen (118).

By analogy with the several infectious diseases of the central nervous system (104-113) in which the oligoclonal IgG bands in the CSF have been shown to be antibodies to specific infectious agents, the finding of similar electrophoretically restricted IgG in 55% of the culture supernatants of RA synovia, and in 17% of RA sera (102), are compatible with a local immune response present in rheumatoid synovitis to a limited antigenic stimulus. Most of the 55% of the RA synovial culture supernatants with oligoclonal IgG showed several closely spaced IEF bands. Previous studies of highly purified human myeloma IgG, which contained a single light chain and idiotype, have shown electrophoretic microheterogeneity, similar to that observed in the RA synovial IgG, with 3 to 6 closely spaced IEF bands which possess slightly different isoelectric points. The basis of the slight differences in migration of bands from a myeloma IgG probably results from post-translational changes in the IgG molecule induced by oxidation, glycosylation, deamination or tertiary structural modifications. Since synovial tissues contain many macrophage-like cells rich in proteases, peroxidases and other enzymes capable of modifying IgG, it was considered that some of the microheterogeneity of the IEF bands of synovial IgG might represent such changes.

However, no substantial change in pI, or in this microheterogeneity in IEF occurs during the 18-hour culture period when exogenous 125-I-labeled IgG of the four IgG subclasses was added to the RA synovial cultures. Thus, the observed IgG banding in IEF noted in the RA synovial culture supernatants would not appear to be the result of degradation during the period of culture.

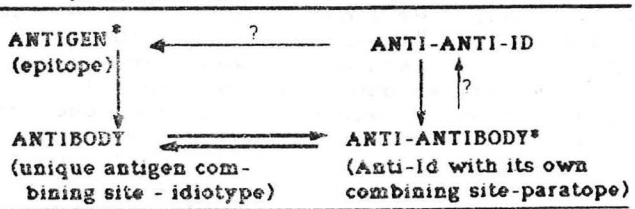
As shown in Figs. 3,4, and 5, several restrictive IEF banding patterns were found in RA serum and synovial IgG. The schematic representation of the IEF bands showing increased amounts of IgG from RA patients (Fig. 5E and 5F) illustrate the two banding patterns found in most RA sera. The IgG of two RA sera from patients donating synovia, and 13 of the 16 other clinically active RA patients' serum IgG contained one or more of these 6 IEF bands (bands 2,3,4,5,6, and 9 of Fig. 5). All of the major bands seen in the 6 RA synovial culture supernatants were also found in the IgG from the serum of the other clinically active RA patients showing IEF banding.

Is it possible that the limited number of IEF bands of IgG noted in the synovial culture supernatants represent a shared immune response to a common antigen in RA? Recent IEF studies of human anti-diphtheria toxoid antisera (116) have shown sharing of IEF spectrotypes of IgG antibodies to toxoid Fragment A, with this IgG sharing the same subclass and light chain type among multiple unrelated individuals. Thus, the limited number of IEF bands of IgG found in the RA synovial culture supernatants might be explained by a shared common antigen which elicits a slightly different immune response from one patient to another.

Although at this point, insufficient data are available to resolve this question, the availability of oligoclonal synovial IgG isolated from bands cut from agarose gels after isoelectric focusing provides a unique opportunity to gain more information about the stimulating antigen(s) in RA. Because each antibody to a specific antigenic determinant has a unique structure in its antigen-combining site (idiotope), and this idiotope, in turn, can be used to elicit antibodies against it in other animals (anti-idiotype), reagents can be generated which allow selective isolation of the original antibody from the polyclonal Ig-mass present in serum or body fluids. This approach has been used to study the biology of a human B cell lymphoma, and even to eliminate lymphoma cells in culture (119), and to identify a specific viral agent reactive with an oligoclonal IgG band from a patient with multiple sclerosis (120). The current availability of human hybrid myeloma cells which will allow the creation of human-human monoclonal antibody reagents (121) makes the use of monoclonal anti-idiotypic antibodies against RA synovial IgG idiotopes (to selectively immunosuppress RA) a realistic future possibility. The rationale for this approach is shown in Table VII.

TABLE VII. IDIOTYPE: ANTI-IDIOTYPE NETWORK FOR THE REGULATION OF ANTIBODY PRODUCTION

JERNE. ANNALS IMMUNLOLOGY PARIS. 1974



* Antigen and anti-Id= Homobodies with the epitope and paratope sharing a similar charge distribution

SPECULATIONS REGARDING POTENTIAL ANTIGENS STIMULATING THE SYNOVIUM

IgG STIMULATING RHEUMATOID FACTOR PRODUCTION?

In the past, it has been assumed that most of the IgG made by the RA synovium represented IgG rheumatoid factor (anti-IgG). However, our previous studies have shown an average of only 5% of the F(ab')2 fragment of IgG isolated from RA synovial cultures to combine with aggregated IgG (122), and an average of only 9% of synovial IgG to show internal cyclization compatible with IgG rheumatoid factor (101,123), as assessed by the relatively insensitive technique of separation by Sephadex G-200 chromatography. This low percentage of synovial IgG with rheumatoid factor specificity recently has been supported by studies of rheumatoid factor production by isolated lymphoid cells eluted from RA synovial tissues (124).

VIRAL OR BACTERIAL INFECTION OF RA SYNOVIUM?

Just when physicians become smug about a given disease having no infectious etiology, Nature rises up and swats them another surprise. The recent surprises of Legionnella, and the Borellia spirochete in Lyme arthritis are acute reminders that some organisms are difficult to see within the tissues which they infect. Even less can be said about detection of viral agents during their "eclipsed" phase of cellular infection. For this reason, obligate, intracellular bacteria or viral agents remain very real possibilities as causative agents in RA. Some suggestive evidence supports the possibility of Epstein-Barr virus as such an agent. Recently, the sera of RA patients has been shown to contain antibodies which identify multiple Epstein-Barr virus-induced nuclear antigens (125), although the Epstein-Barr viral genome cannot be demonstrated in DNA isolated from RA synovial tissues (4). Although mycoplasma agents cause well-documented arthritis in animals, as yet, no consistent isolation has been obtained from rheumatoid joint tissues or RA synovial fluid (126). The occasional isolation from a synovial biopsy of an organism causing arthritis even after negative blood and synovial fluid cultures (127) should generate caution about negative isolation results, however.

Muramic acid derived from bacterial cell walls has been shown to cause chronic erosive arthritis in rats resembling RA, even after viable organisms cannot be isolated. Muramic acid deposits can be eliminated from the joint and the arthritis stopped by infusion of the enzyme "mutanolysin" (128). To evaluate the possibility that RA could have this type of immune stimulus, Bennett, et al (129), developed a sensitive mass spectrometric procedure for the detection of bacterial cell wall components in rheumatoid joint tissues, but could detect none.

AN ENTEROPATHIC BASIS FOR RHEUMATOID ARTHRITIS?

Since Virchow, man has been suspicious that the mass of bacteria carried around in his colon may cause him illness, and has sought to manipulate his enteric environment by dietary changes. Recently, the fasting of patients with RA has gained popularity, although the usually-associated bed rest has often been given the credit for any changes in clinical activity. Sundqvist, et al (130) evaluated the impact of fasting on both joint score and intestinal permeability in 5 RA patients. They found significant improvement in joint score associated with a drop in intestinal permeability to low molecular weight polyethylene glycols (PEG 400) when the patients were fasted

for 10 days. Five non-fasted RA patients otherwise treated in the same way, did not show significant changes in either parameter. When the fasted patients were then given a lactovegetarian diet, all changes reversed to pre-fasting levels. All treated and control patients were given laxatives and enemas prior to the fasting period (130).

The role of diet and its impact on intestinal flora in causing RA has also been studied. By feeding a high protein diet of 20% fish meal, Mansson, et al (131), produced a chronic, erosive polyarthritis in young pigs which was clinically similar to RA. The piglets showed a huge increase in Clostridium perfringens in their feces and a large rise in serum antibodies to C. perfringens. Pigs fed a normal grain diet did not develop arthritis, nor did they show any change in intestinal flora or increase in antibodies to C. perfringens. Involved animals showed significantly elevated ESR, hypergamma-globulinemia, and their joint tissues showed histopathological features similar to those of RA. The WBC of synovial fluid averaged 15,000/cu mm, 80% PMNs. The synovial membrane showed lymphoid cell infiltration, villous hypertrophy, pannus formation and produced cartilage and bone erosions identical to those found in RA. Most of the pigs developed subcutaneous nodules, and showed an increase (when compared to control pigs) in lymphoid cell infiltration of Peyer's patches in the ileum, but no other distinctive intestinal abnormalities were observed. Pigs fed a bacteriologically sterile protein diet still developed an increase in C. perfringens in their feces. Unlike RA, none of the arthritic pigs developed rheumatoid factor, and the arthritis was prevented by simultaneously feeding the pigs zinc. The authors interpreted the zinc effect as, "altering intestinal permeability in some manner". Cultures of inflammed joint tissues were sterile and no other unusual bacteria were cultured from the feces. Mansson and Olhagen (132) later showed that 67% of patients with RA have increased C. perfringens in their feces while only 0.9% of healthy controls harbored this organism in detectable levels. They also found C. perfringens in the upper jejunum in some RA patients.

The support for antigen from the intestine flaring arthritis was provided by the finding that experimental immune arthritis in mice could be activated after challenge with oral antigen (133). The recent documentation of two cases of RA which began after intestinal bypass surgery (134,135) also stimulates interest in an altered intestinal permeability as the basis for foreign antigen exposure and arthritis activation. The principal immunologic barrier to antigens in the gastrointestinal tract entering the general circulation is the secretory IgA (S-IgA) system. IgA is produced by 85% of the plasma cells in the intestinal wall, and any genetic or acquired defect in this system would allow access of antigen. Russell, Brown and Mestecky (136,137,138) have shown that a hepatobiliary recirculation of S-IgA occurs, and that bile contains more IgA than any other body fluid. Parenchymal cells of the liver have a specific receptor for the secretory component of S-IgA, and thus remove immune complexes coming from the gut, preventing them from entering the general circulation. A defect in the S-IgA recirculation or excess antigen load might allow gut antigens to enter the general circulation and elicit an IgG response leading to IgG-immune complexes with predilection for the joint, and stimulating anti-IgG (rheumatoid factor) production.

IMPACT OF THERAPY ON IMMUNE COMPONENTS OF RA SYNOVITIS

The treatment of RA is big business. Over $1.3 billion dollars are spent annually for non-steroidal anti-inflammatory drugs alone. Estimates of the burden of RA on medical care, both outpatient and inpatient, has placed it at 5% of all medical dollars spent. When one adds to this the lost wages, pain, crippling and increase mortality (6) generated by RA, it is easy to understand the pressure on internists, orthopedic surgeons and others to develop more effective means of treatment.

Aspirin and the several non-steroidal anti-inflammatory drugs produce pain relief and reduce joint heat, redness and swelling, and even lower the ESR. Unfortunately, none of them alter the cartilage, bone and tendon destruction produced by RA synovitis. This says a lot about the role of prostaglandins as primary agents in inducing the activation of the various immune cells in the rheumatoid synovium, because most prostaglandin release is blocked by these agents.

Most of this portion of the discussion will, therefore, be directed at the Phase 2 drugs capable of inducing a remission in the synovitis (See Table VIII). Gold compounds are the oldest, and at present, the most efficacious of these agents. Recently Lipsky, et al (139,140) have suggested that gold alters the way in which the monocyte-macrophage population functions, thus changing the cell-cell interactions with T-and B-lymphocytes in the rheumatoid synovium. However, about 25% of RA patients develop toxicity to gold (141, 142), and another 15% do not respond to a full 20-week course of treatment. Yet another 15% relapse while on gold therapy, leaving only about 45% of all RA patients started on gold with a beneficial outcome. The Mayo Clinic Rheumatology Section (144) has attempted to find genetic and other factors which might predict which RA patients will respond, and which will not respond to gold treatment. Of 54 patients, only 45 were able to complete a minimum of 6 months of gold therapy. Five of the 9 patients dropping out had gold toxicity, 1 had a myocardial infarction, and the other 3 elected to quit the study for non-medical causes. Of the 45, 18 were not significantly improved, 18 others were definitely improved, and 9 patients were markedly improved. HLA-A3 positivity and HLA-DR4 negativity were the two single best predictors of a good response. Patients mildly anemic (mean Hgb = 11.8) did better than patients not anemic (mean Hgb = 13.0). Patients in the first two years of illness had a greater chance of marked improvement than patients with RA for more than 3 years. In another study (144) a low serum IgA, both before starting gold and after 3 months of treatment correlated with the appearence of gold-induced toxicity.

TABLE VIII. TREATMENT OF RHEUMATOID ARTHRITIS
PHASE 2 - DRUGS INDUCING REMISSION

AGENT	DOSAGE	TRIAL PERIOD	% TOXICITY	% SUCCESS
GOLD THIOMALATE THIOGLUCOSE	50 mg/ week	20 weeks	25%	60%
PENICILLAMINE	750 mg/day	20 weeks	33%	50%
AZULFIDINE	3000 mg/day	20 weeks	20%	UNKNOWN
CAPTOPRIL	200 mg/day	20 weeks	35%	UNKNOWN

TABLE IX. MORE COMMON REACTIONS TO GOLD

(FROM DORWART, ET AL, ARTH RHEUM 21:513, 1978)

TYPE OF REACTION	RHEUMATOID ARTHRITIS (42)		PSORIATIC ARTHRITIS (14)	
SKIN RASH				
MILD	5%		-	
SEVERE	17*		7%	
PROTEINURIA	5	*	21	*
STOMATITIS		26.2%		14.3
MILD	5	stopped	-	stopped
SEVERE	5*	gold	3*	gold
LEUKOPENIA	6	Rx	5	Rx
THROMBOCYTOPENIA	3		3	

If gold treatment of RA is not tolerated or is not effective, what should be the next step? Most rheumatologists would consider treating patients with severe RA with D-penicillamine. Although equally efficacious, D-penicillamine is more toxic than gold with approximately 35% of patients showing toxicity sufficient to require withdrawal of the drug (145). The more common reactions to D-penicillamine are shown in Table X.

TABLE X. MORE COMMON REACTIONS TO PENICILLAMINE

(FROM DOUBLE-BLIND TRIAL, ANDREWS, LANCET 1:275, 1973)

REACTION	RA Patients PENICILLAMINE	RA Patient CONTROLS
Impaired Taste	25.5%	8%
Thrombocytopenia	21.6	0
Proteinuria	7.8	0
GI Upset or Nausea	31.4	23.5
Rash or Mouth Ulcers	23.5	21.6
Total Stopping Drug after 1 Yr	35.0%	

TABLE XI. USE OF PENICILLAMINE IN RA PATIENTS NOT RESPONDING OR ALLERGIC TO GOLD

(FROM ROTHERMILCH, N., 1979) 200 patients

Patient Characteristic	Percent
Had prior gold toxicity	36.5
Penicillamine toxicity	31.5
Toxicity to both drugs (12 same toxicity to both drugs)	14.5
No benefit or relapse both drugs	13.0

About 10% of patients with RA complete gold and D-penicillamine, and develop allergies or other toxicity to both, or do not respond to either agent. These patients pose a particularly difficult decision for the physician. Some have mild enough disease that low doses of prednisone (5 to 7.5 mg/day) pose less long-term risk than immunosuppressive drugs. Others might be considered for milder remittive agents of low or unproven efficacy such as hydroxychloroquin, 5-thiopyridoxine (147), captopril (148), or Azulfidine (sulfasalazine) (149,150). The last three of these are still in the experimental phase, although only the 5-thiopyridoxine is not yet released for other therapeutic purposes.

However, more severe patients, particularly those with vasculitis or extraarticular complications of RA should be considered for Phase 3 treatment. Of these Phase 3 drugs, only prednisone and azathiaprine (146) are officially approved by the Food and Drug Administration for use in RA.

TABLE XII. TREATMENT OF RHEUMATOID ARTHRITIS
PHASE 3 IMMUNOSUPPRESSIVE DRUGS

AGENT	DOSAGE (mg/day)	TRIAL PERIOD	% TOXICITY	% SUCCESS
Prednisone	5-10	2-10 wks	30+	?
Methotrexate	7.5-20* *per wk	8-20 wks	50	50
Azathiaprine	75-200	8-20 wks	50	50
Cytoxan	50-200	8-20 wks	70	70
Chlorambucil	2-8	8-20 wks	70	70

The very good results of oral and intramuscular methotrexate offer the most promise for patients with otherwise medically-refractory RA at this time. The quick reversability of the effect of methotrexate with oral folinic acid makes it more attractive for use than alkylating agents such as cyclophosphamide (151), and unlike azathiaprine and alkalating agents, methotrexate is not thought to increase future risk of neoplasia. Thoracic duct drainage (152), leukopheresis and plasmapheresis (153) and intraarticular (154) or total body radiation of highly selected patients with severe RA all remain experimental at this time.

TABLE XIII. INTRAMUSCULAR PULSE METHOTREXATE FOR TREATMENT OF SEVERE RA

(ANDERSEN, WEST, AND CLAYPOOL, ARTH RHEUM, 1984)

A double-blind, cross-over study of 10-20 mg of MTX im per week versus placebo therapy in 15 RA pts.

Patients followed for 13 wks, 1st phase, then switched.

MTX treated patients did better than placebo treated patients in joint scores, pt global assessment, walking time, AM stiffness, ESR (all $p=< 0.05$), and in doctors' assessment ($p=<0.01$). No improvement during placebo period and patients worse when switched from MTX.

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