A NEW LOOK AT CIRCULATING IMMUNE COMPLEXES IN RHEUMATOID ARTHRITIS

INTERNAL MEDICINE GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER AT DALLAS
J. DONALD SMILEY, MD
JUNE 20, 1996

A New Look at Circulating Immune Complexes in Rheumatoid Arthritis

For many years immune complexes (IC) have been seen as a potential source of specific antibodies and antigen(s) which could unravel the jigsaw puzzle of rheumatoid arthritis (RA) and other diseases with an immunologic pathogenesis. In the 1970's and 1980's, useful methods for measuring IC, such as the Raji cell binding assay, bovine conglutinin binding, $F(ab')_2$-anti-C3 binding (1,2), monoclonal rheumatoid factor binding (3), and C1q-binding (4) were developed and widely tested with inconsistent and usually disappointing results (3,4). With the possible exception of anti-DNA:DNA IC in SLE sera (5,6), very little correlation between serum levels of IC and clinical parameters of disease activity could be made (7). Some puzzling results emerged which led investigators to abandon most IC research for more fruitful areas. For example, Pereira, Theophilopoulos and Dixon (8) found evidence for C3-fragments bound to monomeric IgG in the serum of patients with RA (Fig 1). These low molecular weight IgG-C3 "IC" varied widely from one patient to another, and no identifiable antigen could be demonstrated (9,10). (See Fig. 1)

At that point in time, many aspects of complement activity and its role in IC processing was not known. The ability of C3 and C4 to form covalent bonds with antibody (and antigen?) via a reactive thioester bond (11) or its acyl-imidazole derivative (12) (See Figure 2) was not understood. We now know that there are two forms of C4 derived from two distinct genes (C4A and C4B) that code for separate forms of C4 which differ in a few amino acids, but that the thioester bond in the C4A gene product reacts with amino residues to form an amide bond. The C4B-gene product has a thioester bond which, when activated, initially reacts with an adjacent histidine to make a reactive acyl-imidazole intermediate that selectively reacts with hydroxyl residues to form ester bonds (12). It is highly probable that C3 also follows the path of the C4B-gene product to form covalent ester bonds with hydroxyl residues on the target IC (and adjacent proteins or carbohydrates) (12).

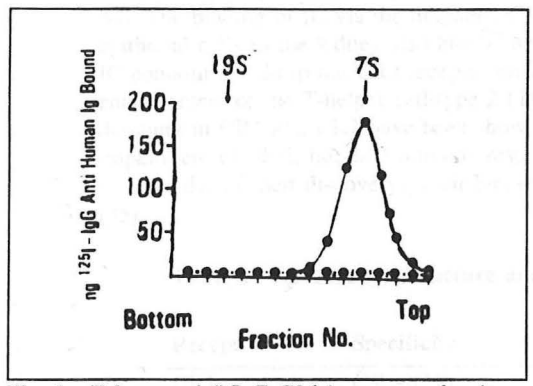

Fig. 1. "Monomeric" IgG-C3d in sucrose-density-gradient fractions, detected with polyclonal anti-C3 antiserum. (Ref. 8).

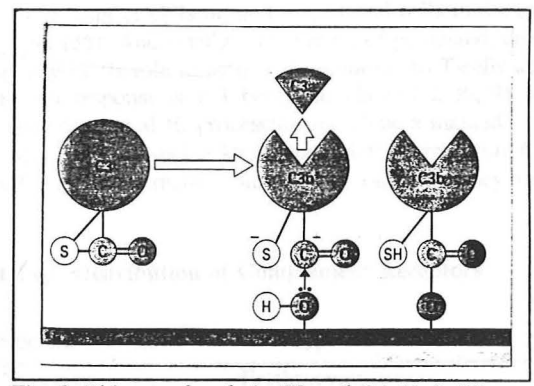

Fig. 2. Thioester bonds in C3 and C4 (Ref. 13).

Once formed, the covalent ester or amide bonds of C3 and C4 are very strong, and allow an attachment site for complement receptors (CR1 and CR3), which are involved in processing IC. Once the C3-component of IC has been degraded to iC3b/C3d, it can be bound to the CR2 receptor on B lymphocytes, allowing antigen presentation to T-cells eventually stimulating a selected humoral immune response (12). These ester bonds, but not the amide bond from C4A-derived C4, can be cleaved *in vitro* with alkaline hydroxylamine to identify the site of complement attachment to antibody or antigen (14).

Whether classical, alternative or other pathway activation of complement occurs depends on variations in composition of different IC. The presence of different subclasses of IgG (15) or IgM antibody; the antigen:antibody molar ratio (16); the presence of glycosylation of the IgG antibody (17-27)

(See Fig. 3); and/or the presence of IgA or IgM co-precipitation with the IgG in the IC (28-30) all determine how much C3 and C4 will be bound to a given IC. The presence of complement deficiencies, inherited or acquired, and the chemical nature of the antigen also influence the degree and rapidity of C3 C4 interaction with a given IC (31).

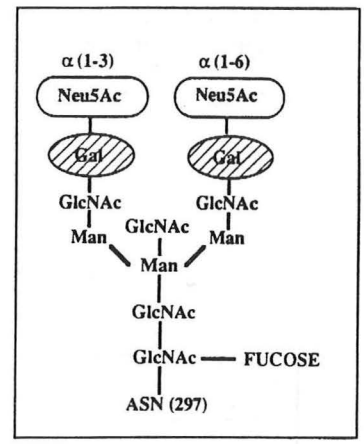

Fig. 3. Biantennary complex oligosaccharide of the Fc region of human serum IgG (From Axford, JS, *et al., J. Clin. Invest.* 89:1023, 1992).

Neu5Ac = sialic acid
Gal = galactose
GlcNAc = N-acetylglucosamine
Man = mannose
ASN = Asparagine

Much information has been obtained about the mechanisms of binding of IC to the complement receptor-1 (CR1) on erythrocytes (RBC's), and polymorphonuclear leukocytes (PMN's) via C3b and C4b 34). The binding of IC via the degraded C3b (iC3b) to Kupffer cells in the liver, littoral cells in the splee epithelial cells in the kidney also has been worked out (35). And finally, the binding of processed, degra IC containing C3d to the CR2 receptor on B-lymphocytes, its role in antigen presentation to T-cells and enhancement of the T-helper cell-type 2 (TH2) immune response is now being elucidated (12,36,37). Mic deficient in CR1 and CR2 have been shown incapable of normal IC processing and show a marked impairment of their humoral immune response (38). Table 1 lists the known complement receptors (nan in the order of their discovery), their binding specificity, their structure and the cells on which they are f (35).

Table 1. Specificity, Structure and Cell Type Distribution of Complement Receptors

Receptor	Specificity	Structure	Cell Type Distribution
CR1	C3b>iC3b	4 allotypes: 160K, 190K 220K, 250K	Erythrocytes Granulocytes, Monocytes Kidney podocytes Dendritic reticulum cells
CR2	iC3b=C3dg	140K	B Lymphocytes
CR3	iC3b	$\alpha_m\beta_2$Integrin 165K α-chain 95K β-chain	Macrophages, Kupffer cells Monocytes, granulocytes NK cells
CR4	iC3b=C3dg	CD11c/CD18 150K α-chain	Monocytes, Macrophages Granulocytes

CR1 on RBC's has been studied in detail in both normal and disease states (39-41) (See Fig. 4). There is considerable variation from person to person. Between 170 and 1060 CR1 chains per RBC have been detected (40) (See Fig. 5). These are not spread uniformly over the cell surface, but rather are clustered around cell membrane pits so that they can bind an IC in concert to create a much stronger bond ($K_a=10^{-11}M$) when compared to the binding of a single CR1 chain ($K_a=10^{-6}M$) (42) (See Fig 6 on next page). Although PMN's and some macrophages also have CR1 receptors, these are not bunched together, but are scattered over the cell membrane as single chains (possibly capable of capping if enough time in contact with an IC is available) (42).

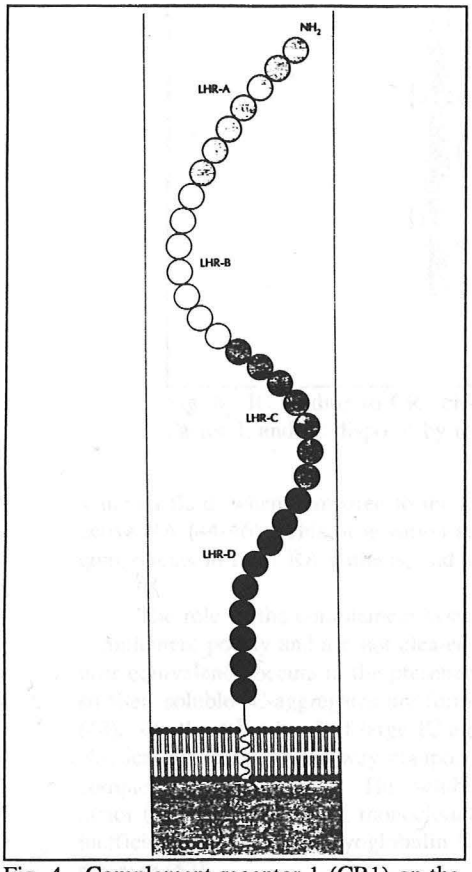

Fig. 4. Complement receptor-1 (CR1) on the erythrocyte membrane (Fearon, DT, *Hospital Practice* 23:63-72, 1988).

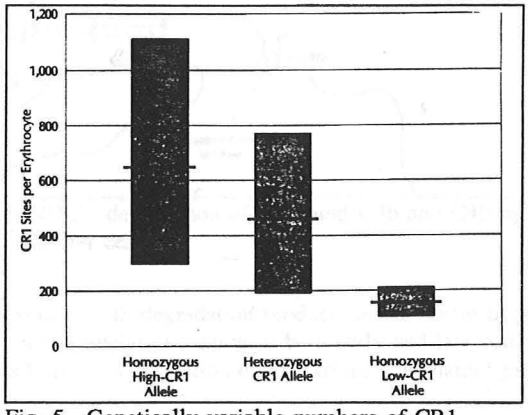

Fig. 5. Genetically variable numbers of CR1 receptors on human erythrocytes (From Fearon, DT, Ref 39).

There is indirect evidence of complement activation, especially in the joint in active RA, in the form of much lower complement component levels in synovial fluid than in the serum of the same patient in almost every instance (43). Rheumatoid factor positive patients have the lowest levels (43), but even seronegative patients also show low synovial fluid C3 levels. Most RA patients have normal or even elevated

complement component levels in their serum. There is simultaneous and parallel elevation of the complement degradation products, C3dg and the fluid-phase terminal complement complex (SC5b-C9) in

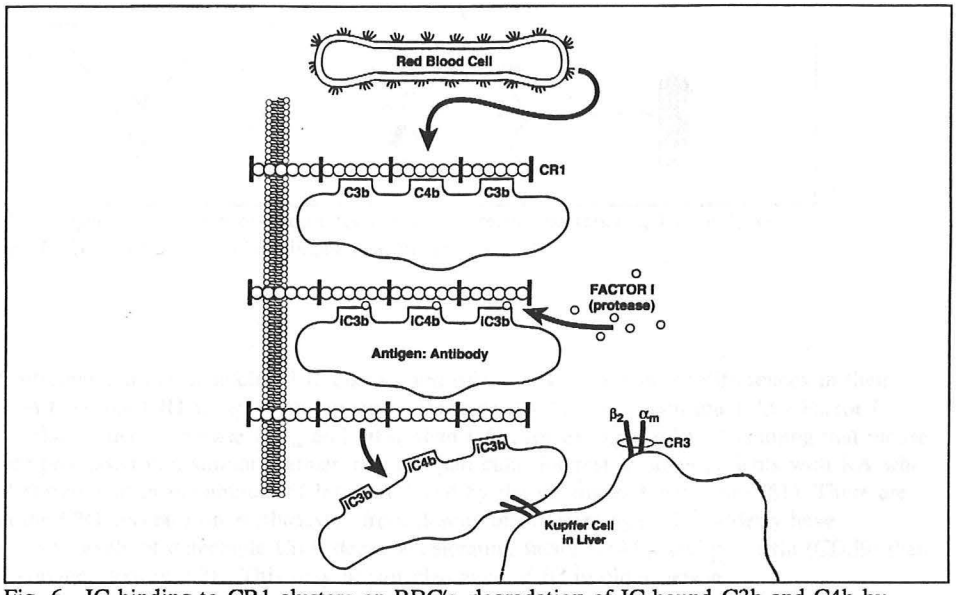

Fig. 6. IC binding to CR1-clusters on RBC's, degradation of IC-bound C3b and C4b by Factor I, and IC disposal by the liver Kupffer cells.

synovial fluid, when compared to the lower levels of both degradation products in the serum of patients v active RA (44-46). This observation supports intraarticular activation of both early and late complement components in most RA patients, and suggests infrequent activation outside of the rheumatoid joint.

The role of the complement system in the handling of IC is multifaceted. Antigen-excess IC bind complement poorly and are not cleared by the erythrocyte CR1 receptor (47). If antigen:antibody interact near equivalence occurs in the presence of adequate classical complement pathway components, much smaller, soluble IC-aggregates are formed (48) and these are readily bound by the erythrocyte CR1 recep (48). On the other hand, if large IC-aggregates are formed in the absence of adequate amounts of the classical complement pathway components, these larger IC can later be solubilized by the alternative complement pathway (49). This solubilization is markedly diminished by the presence of IgM rheumatoi factor (50,51). In addition, monoclonal IgG rheumatoid factor (which dimerizes with itself) fixes C3 and inefficiently, and mixed cryoglobulin IC made up of IgM-RF:IgG-RF is not cleared by the erythrocyte C1 receptor (52).

The mechanism of release of C3b/C4b-IC from the CR1 receptor on erythrocytes has been extensiv studied, and it is now known that the CR1 receptor acts as a cofactor for the action of an unusual serine proteinase, Factor I, (formerly known as C3b inactivator), to convert C3b to iC3b, then to C3d (53) (See 7 on the next page). The iC3b remains bound to the CR1 receptor, albeit with much lower affinity, but C is not bound by CR1 (54). This release by Factor I is inhibited up to 90% by the presence of 10 millimo $ZnCl_2$ (55). In the rare individuals in whom Factor I is genetically absent (56), susceptibility to pyogenic infections (57) and a multisystem vasculitic disease (57-59) has been observed.

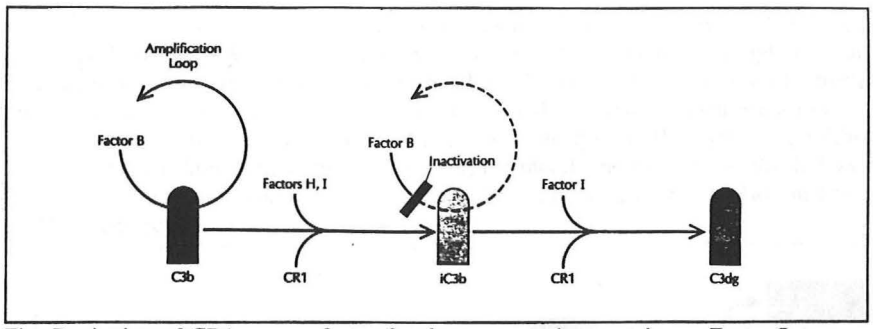

Fig. 7. Action of CR1 as a co-factor for the serum serine proteinase, Factor I, to degrade IC-bound C3b and C4b (From Fearon, DT, Ref. 39).

IC made of different murine monoclonal Ig classes and subclasses show marked differences in their ability to be released from the CR1 receptor on human erythrocytes by Factor I, with much less Factor I (<5% of normal levels) needed to release IgG_{2a} and IgG_{2b} than IgG_3, for example (60). Assuming that mouse and human IgG_3 are processed in a similar manner, this has particular interest in some patients with RA who show a substantial enrichment in the amount of IgG_3 produced by the rheumatoid synovium (61). There are also differences in the CR1 receptor on erythrocytes from donors of different ages. The elderly have erythrocytes with lower levels of detectable CR1, decay accelerating factor (DAF), and protectin (CD59) than erythrocytes from younger donors (62). This may impair clearance of IC in older persons.

The dynamics of the transport and elimination of IC by the CR1 receptor on erythrocytes was beautifully illustrated by the study of Cornacoff, et al, (63) (See Fig. 8).

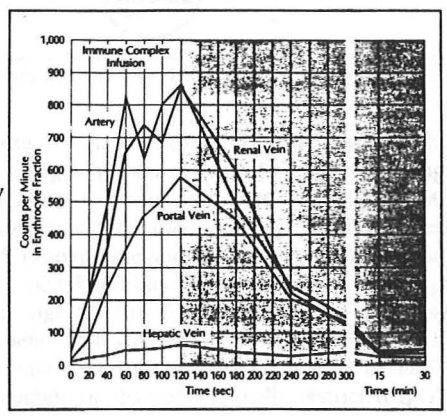

Fig. 8. Single passage clearance of human dsDNA:anti-DNA IC from red blood cells by the liver (Cornacoff, JB, *et al, J Clin Invest* 71:236, 1983).

Using radioactively labeled human immune complexes in a baboon model, they were able to show single passage clearance of IC from red cells by the liver, with minimal clearance by other organs such as kidney or spleen. The exact mechanism by which this occurs is not established, but probably depends on the CR3 receptors on Kupffer cells which have a higher affinity for iC3b than does the CR1 receptor on the red cell, and also, perhaps, on the very high blood flow to the liver when compared to the spleen. A second study of the release of dsDNA:anti-DNA IC was carried out *in vitro* by Taylor, et al, (64). In clotted whole blood,

even after two hours, very little of the bound IC was released. Slightly more IC was released into plasm anticoagulated with EDTA, but that study made the very strong point that simple measurement of serum without appropriate analysis of the fraction bound to CR1 on erythrocytes could be misleading and completely underestimate the amount of IC being cleared in a given clinical situation.

In RA, the traffic of IC from the joint requires an analysis of the dynamic equilibria which exists *i vivo* among at least three compartments: the joint, the blood, and the liver and/or kidney. In addition, th phagocytic cell population within the joint, and in lymph nodes and spleen also must be considered for tl role. These compartments are outlined in Fig. 9.

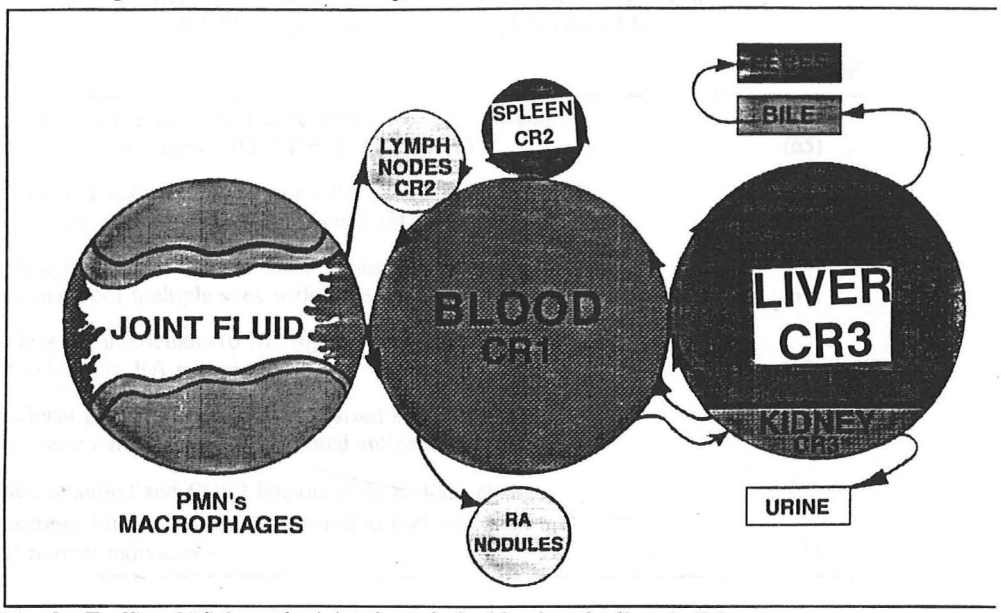

Fig. 9. Traffic of IC from the joint through the blood to the liver in RA

Although subject to considerable debate, the analysis of the T-lymphocytes infiltrating the rheumatc synovium, especially in patients in the early stages of their disease (65), has increasingly suggested that t immune response in RA is not random, but rather is driven by a restricted antigenic stimulus (65-67). Th impression has been reinforced by the demonstration that there is a high level of interleukin-10 (IL-10) being secreted by the T cells within the RA synovial membrane, completely unlike the cytokine profile b released by circulating T lymphocytes in the peripheral blood (68). Since IL-10 is a potent suppressor of synthesis of proinflammatory cytokines, this high level of IL-10 secretion by joint tissues might explain t poor proliferative response of T cells in patients with RA (68). This difference in IL-10 level also indicat distinctive population of T cells within the joint compared to those circulating in the blood. This presentation will assume that the large production of electrophoretically-restricted IgG, IgA and IgM with the RA joint (69,70) is antigen-driven (71), and that the high-molecular weight IC found in the synovial fluid of patients with joint effusions represents the combination of the locally-produced Ig with this antigen(s), followed by complement fixation, and the addition of rheumatoid factor. If the antigen has be processed by macrophages, and released as "indigestible" fragments of relatively small size, the proportio antigen in the immune complex could be very low (in the order of 0.5-1% of the total IC weight). This makes detection particularly difficult if crude IC isolation procedures are used such as polyethylene glycc precipitation. The accumulating evidence that the immune response in the RA joint is antigen-driven is shown in Table 2 on the next page.

The difficulty of the isolation problem of IC from synovial fluid, and especially from the blood of

patients with RA has been lessened by the development of monoclonal antibodies specific for neoantigens generated by the degradation of C3b which is covalently attached to most IgG- and IgM-containing IC. Tamerius, Pangburn and Muller-Eberhard, and their coworkers (72-74), and Garred, Mollnes, Lea and Lachmann (75) have developed solid-phase binding immunoassays for iC3b/C3d containing IC, and these have been applied (76,77) to quantitate C3-fragment-containing IC in RA. Others have used polyclonal antibodies against C3d to quantitate IC in synovial fluids from patients with juvenile rheumatoid arthritis (78). (See Fig 10 on the next page.)

Table 2. DATA SUGGESTING THAT THE IMMUNE RESPONSE IN THE RA JOINT IS ANTIGEN-DRIVEN

Finding	*References*
Decreased heterogeneity of T cell antigen receptor V_α and V_β transcripts in synovial fluid T cells in early RA	(65)
Oligoclonal T cell populations from RA synovium propagated in severe combined immunodeficient (SCID) mice	(66)
High frequencies of identical T cell clonotypes in RA synovial tissues from multiple sites within the RA joint	(67)
High level of interleukin-10 (IL-10) production by activated T cells from RA synovium	(68)
Oligoclonal population of IgG synthesized by RA synovium in tissue culture suggesting limited antigenic stimulation	(69,70)
Somatic mutation and CDR3 lengths of Ig κ light chains expressed in RA patients compared to κ-chains from IgG of normal individuals	(71)

Work by Dr. Wayne Hoffman and his coworkers (79) at the Arthritis Research Laboratory at Presbyterian Hospital has shown that levels of IC in separated serum does not correlate with disease activity of RA because blood IC are cleared from the serum by the CR1 on red blood cells (RBC) for transport to the liver. They showed that IC were released from the RBC of RA patients by *in vitro* incubation of clotted blood at 37° C. See Fig 11a-d on the next page. The released IC were detected by an ELISA method using mouse monoclonal antibody against a neoepitope on the C3d fragment of C3 (which is covalently bound to the IC) as the capture antibody, and biotin-labeled, $F(ab')_2$ fragments of mouse antibody against the Fc portion of IgG to detect IgG-IC. Streptavidin-conjugated horseradish peroxidase is then used to complete the ELISA assay and create a blue color which can be quantitated spectrophotometrically (Fig 10).

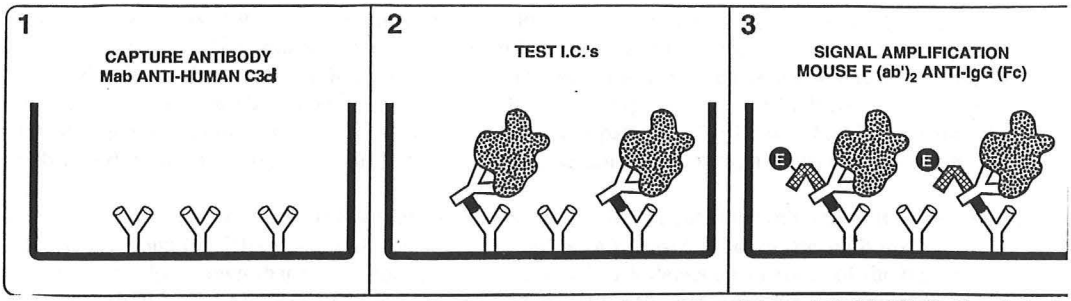

Fig. 10. ELISA technique used to detect IgG-IC containing fragments of degraded C3 (C3d). Panel A sh the capture monoclonal mouse antibody to C3d attached to the wall of the microtiter plate. Panel B show the addition of serum or synovial fluid fractions containing IgG-IC which contain bound C3d. Panel C shows the amplification phase obtained by adding biotinylated mouse F(ab')2 anti-human IgG which can t be detected with streptavidin-horseradish peroxidase and a peroxide sensitive chemical which produces a blue color.

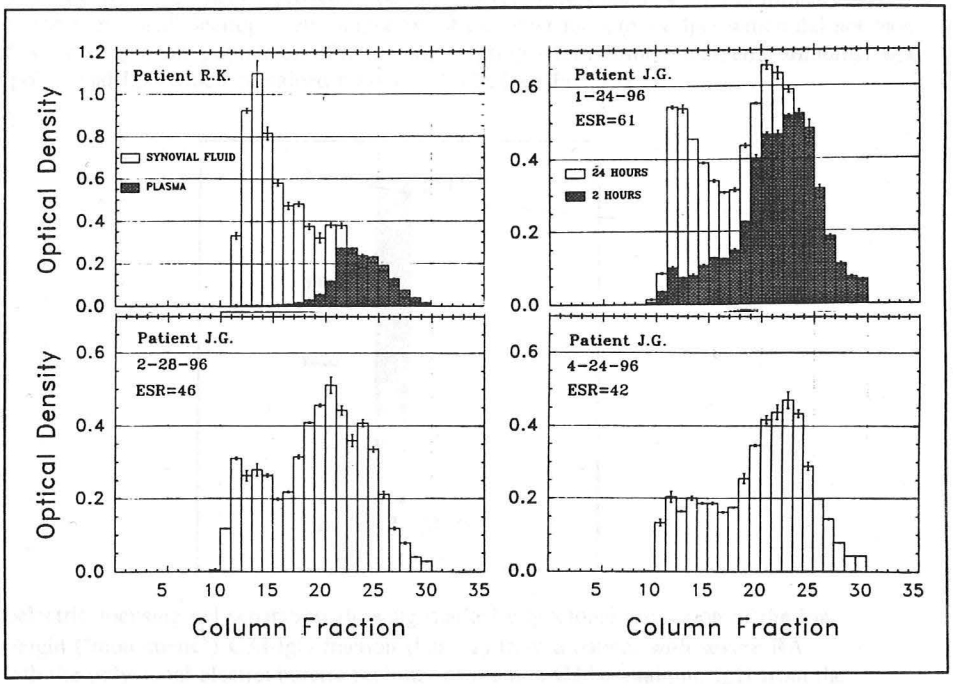

Fig. 11. Fractionation of different-sized IgG-C3d-containing IC on FPLC Superose-6 ® columns. Panel A. From RA synovial fluid and plasma of patient, R.K.; Panel B. From blood serum of RA patient, J.G., separated at 2 hours and 24 hours after standing with the clot containing the erythrocyte fraction at 37°C. Patient, J.G., was begun on 10 mg of methotrexate/week on December 14, 1995. Panels C and D. From blood serum samples from indicated dates of patient, J.G., after 24 hours with the clot.

The size of the released IC was determined by gel filtration and the anti-C3d/ELISA used to quantitate IC levels in each fraction. The results of these studies on RA synovial fluid (Fig. 11a), and on three sequential blood samples from a single RA patient being treated with methotrexate for severe RA (Fig. 11b, c and d) are shown. These studies show that within the RA joint (Fig 11a), IC are high molecular weight and in a much higher concentration than in the serum of the same patient. The IC which spill over into the blood are absorbed on the CR1 receptors on RBC causing the serum levels of high molecular weight IC to be very low.

Analysis of the serum IC from 10 RA patients demonstrated that in patients with active RA, significant amounts of IC are bound to RBC, and when released, the levels of released IC correlate with disease activity. They have also shown that rheumatoid factor (RF) may block detection of some of the high molecular weight (MW)-IC. Analysis of 8 RA synovial fluids (RA-SF) confirmed the large amount of IC in the SF from patients with more severe disease. However, even more high-MW-IC could be detected after incubating the RA-SF at 37° C with normal human serum to provide an additional source of fresh complement components. These findings allow larger amounts of high MW-IC to be isolated for analysis of antigen and antibody components which may be unique to RA.

In addition, Hoffman and his co-workers isolated the "monomeric" IgG fraction from the serum which contained C3d fragments (about 2.5% of the total IgG in a patient with severe RA), and compared its electrophoretic mobility in an isoelectric focusing gel with that of the same patient's IgG which did not contain C3d. The C3d-containing fraction proved to be impressively oligoclonal with 3 dominant bands when compared to the polyclonal electrophoretic migration of the other monomeric IgG which did not have any C3d attached, suggesting a sub-population of antibodies reacting with a limited antigenic stimulus when compared to the polyclonal IgG which contained no bound C3d. (See Fig. 12).

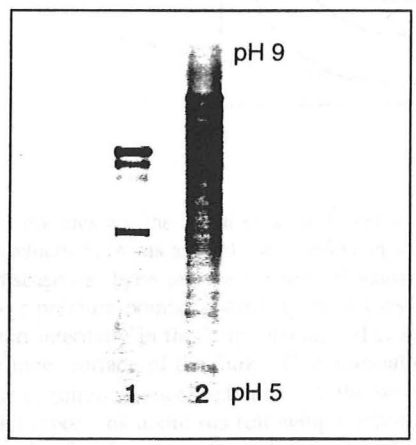

Fig. 12. Isoelectric focusing gel separation showing marked oligoclonal restriction of the low molecular weight ("monomeric") C3d-IgG fraction (Lane 1) from a patient with severe RA compared with the polyclonal electrophoretic mobility of the non-C3d-containing IgG from the same "monomeric" IgG fraction (Lane 2). (Hoffman, WL, AL Camann, and AA Jump, unpublished).

One possible explanation for this unexpected finding would be the release of modified IgG originally part of an IC which had been stripped of antigen within an acidic vacuole within the Kuppfer cell, but which has been recycled back into the blood, still essentially intact and containing the covalently bound fragment of C3. An analysis of the antigenic specificity of this electrophoretically-restricted antibody might

provide important information regarding the antigen driving the RA disease process. These preliminary d suggest that the levels of high MW-IC in circulating blood (mostly bound to the CR1 receptor on the RE and in the synovial fluid correlate with disease activity in RA. Particularly, those patients who are seropositive for rheumatoid factor, have rheumatoid nodules and present with an elevated erythrocyte sedimentation rate (ESR) usually show elevated levels of high MW-IC, but often show variable levels monomeric IgG-C3d, making the latter monomeric "IC" fraction a poor correlate of disease activity.

Between 20 and 45% of RA patients will develop rheumatoid nodules, in part as we shall soon see, related to the widespread use of methotrexate therapy. Rheumatoid nodules appear most often in patients with elevated titers of rheumatoid factor (anti-IgG-Fc), and represent a unique physical marker for RA. They contain an insoluble central core which contains IgG and IgM, fragments of activated complement components, and fibrin as well as cell debris derived from activated macrophages (See Fig. 13).

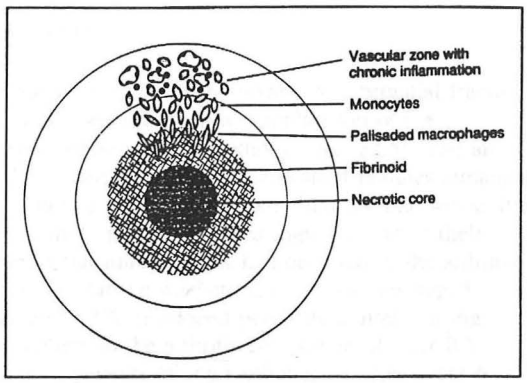

Fig. 13. Diagramatic representation of the structure of a typical rheumatoid nodule (From Ziff, M, *Arth Rheum* 33:761,1990).

I feel that rheumatoid nodules are the result of a spill-over of IC from the usual clearence mechanis either because of excess production, or as a result of a defect in some step in IC elimination. RA nodul usually develop after the disease has been present for several years, and are often first noted over the bo in front of the elbow or over pressure points on the fingers or toes. In some patients, nodules are so extensive that they are found internally in the lung, pleura, other serosal surfaces, within bones (80,81) a even over the brain on the inner surface of the dura. This unusual dissemination of nodules has been termed "nodulosis", and has acquired renewed interest with the widespread use of methotrexate to treat R (82-86). Many of the initial reports of nodulosis following methotrexate represented isolated case reports However, the detailed analysis of 172 patients treated with 10 mg of methotrexate/week for 33 months carried out as a prospective study in France by Combe, et al, (87) has given us an accurate estimate of th risk in patients with RA placed on methotrexate therapy for up to 33 months. Their data (87) are summarized in Table 3 on the next page.

Table 3. NODULOSIS, VASCULITIS AND PERICARDITIS DURING METHOTREXATE (MTX) THERAPY OF RA

Patient Groups Before MTX	No. Pts.	After MTX, 10 mg/week for 4 - 68 months			
		unchanged	improved	worse	%worse
With nodules	40	28	3	9	22.5%
With vasculitis	9	6	0	3	33.3%
Without nodules	132	117	-	15	11.4%
Without vasculitis	132	125	-	7	5.3%
Without pericarditis	132	129	-	3	2.3%

(Modified from Combe, B., *et al.*, *Eur. J. Med* 2:153-156, 1993.)

All of these patients had severe RA which had responded well to methotrexate. A substantial fraction developed rheumatoid nodules and less frequently vasculitis or pericarditis as a complication of the methotrexate therapy. In those patients with nodules present before starting methotrexate, 22.5% had an increase in nodulosis, 7.5% had improvement in their nodules, and in 70%, the rheumatoid nodules remained unchanged. In those patients with digital arteritis prior to starting methotrexate, one third became worse after methotrexate, and two-thirds remained unchanged. None of these patients showed improvement of their digital arteritis after starting methotrexate even though substantial improvement had occurred in the arthritis component of their disease. In patients without nodules before starting methotrexate, 11.4% developed nodules for the first time, 5.3% developed digital arteritis, and 2.3% developed pericarditis after starting methotrexate. All of these patients also had shown improvement in the arthritis component of their RA.

What is not shown is the relatively high drop-out rate of patients taking methotrexate after about 6 months on the drug. This is illustrated in a recently-published, double-blind, controlled study by O'Dell and his colleagues (88) in Omaha, Nebraska, comparing methotrexate alone (10 mg/week), with the combination of sulfasalazine (2 g/day)/hydroxychloroquine (400mg/d)y), and with the combination of all three drugs in these doses . All three groups of RA patients were statistically comparable in terms of disease activity (number of active joints, sedimentation rate, patient's global assessment and physician's global assessment). The number of patients going on with their particular therapy at the end of 9 months and 24 months were compared along with the clinical response parameters. This is shown in Table 4.

Table 4. JOINT SYMPTOMS AND MEASURES OF ACTIVITY IN RA PATIENTS TREATED WITH MTX, HCQ/SSZ OR ALL THREE DRUGS

Variable		MTX 10 mg/wk	SSZ/HCQ	All 3 Drugs	MTX vs 3 Drugs
No.Pts	Base line	36	35	31	p Value
	24 Months	12	14	24	
ESR	Base line	39	45	36	0.63
	24 Months	16	16	10	0.12
Morning Stiffness					
	Base line	190	156	135	0.08
	24 Months	63	50	38	0.34
Tender/Swollen Jts.					
	Base line	31	32/31	29/27	0.58/0.25
	24 Months	7/5	7	3/2	0.06/0.006*
Total Joint Score					
	Base line	63	62	56	0.33
	24 Months	12	14	5	0.007*
Patient's Global Pain					
	Base line	6	6	6	0.59
	24 Months	3	3	2	0.02*
Dr.'s Global Assess.					
	Base line	6	6	6	0.46
	24 Months	2	3	1	0.002*

(Adapted from O'Dell, JR, et al, *N Eng J Med* 334:1290, 1996)
* Statistically significant differences.

As you can see, by the 24th month, the drop-out rate for the 10 mg/week-methotrexate group was 67%! About the same number of patients dropped out of the double-therapy group (SSZ/HCQ), but the triple-therapy group (SSZ/HCQ/MTX) had only 33% of the patients discontinuing treatment, yet all of the efficacy measures favored the triple-therapy group. According to the authors (88), there was no statistically significant difference in toxicity among the three groups of patients, suggesting that most of the drop-outs the first two groups were due to a lack of efficacy. (See Fig. 14).

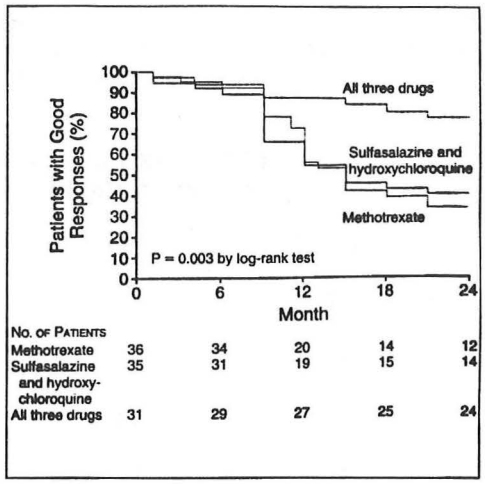

Fig. 14. Patients with good responses to the assigned study treatment. (O'Dell, JR, *et al*, *N Eng J Med* 334:1287, 1996).

Another comparison of "second-line" drugs in RA specifically targeted the question of their impact on the development of rheumatoid nodules (89). This study evaluated 121 RA patients, 55 of whom had, or developed nodules, and assessed the impact of gold, HCQ, SSZ, MTX and D-penicillamine on regression or progression of nodules. The results are presented in Table 5. This study involved a more selected patient population, approximately 45% of whom had or developed RA nodules, but the conclusion that MTX induced nodule worsening or development in a substantial group of patients is inescapable. The distinct possibility that both hydroxychloroquine and sulfasalazine caused regression or disappearance of nodules would also appear likely from this data.

Table 5. PROGRESSION OR REGRESSION OF SUBCUTANEOUS RA NODULES

Nodule Status	GOLD n=30	HCQ n=36	SSZ n=19	MTX n=30	DPEN n=11
No Change	21	19	12	7	10
New or Progression	6 (20%)	4 (11%)	1 (5%)	21 (70%)	1 (9%)
Regression	2 } (10%)	6 } (36%)	4 } (32%)	2 } (7%)	0
Complete Resolution	1	7	2	0	0

(Modified from Bautista, BB, et al, *Arth Rheum* 35:R38,1992)

Several isolated case reports of one to three patients have also reported reversal of methotrexate-induced RA nodules after beginning HCQ-therapy (85,86,90). These reports make the combination therapy with HCQ/SSZ/MTX also look interesting because of the possibility that HCQ/SSZ might give additive therapy which would prevent nodule formation being induced by MTX. Although not extensively studied, azathioprine has also been reported to induce nodulosis in patients with RA (91), again, even though the arthritis component responded to the drug.

In order to explain the paradoxical impact of these various drugs on the arthritis and the rheumatoid nodule in patients with RA, let us take a closer look at what is known about rheumatoid nodules. We now

know that macrophages leave the bloodstream and migrate from the outer rim of a rheumatoid nodule to form the central palisade of cells surrounding the necrotic core (92). These macrophages express HLA-I and CD14 surface proteins indicating activation as antigen-processing and presenting cells (93) (See Tab 6).

Table 6. PERCENT POSITIVE CELLS IN PALISADE, MIDDLE, AND VASCULAR ZONES OF 14 RHEUMATOID NODULES

Monoclonal Ab to	Palisade	Middle	Vascular
Leukocyte Common Antigen	97	91	55
Monocytes	87	81	56
Active Macrophages	98	81	34
Complement Receptor 3	98	89	55
HLA-Class II (DR+)	95	95	95

(From Palmer, et al, *Arth Rheum* 30:729, 1987)

The central necrotic core of the nodule contains very little scar tissue (collagen) (94), but does cont fibrin and along with IgG, IgM, and complement components (95,96), compatible with insoluble immu complexes entrapped in fibrin. (See Table 7).

Table 7. EXTRACELLULAR DEPOSITS IN SECTIONS OF RA NODULES

Antibody to	Central Necrotic	Palisading Area	Peripheral Granuloma
IgG IgA, IgM	Diffuse +	0	0
C3c	Diffuse +	0	2-3+ in sm. vessels
C5b-C9	Diffuse +	0	1-2+ in sm. vessels
Fibrin	Diffuse 3+	0	3+ in vessel walls

(From Mellbye,OJ, et al, *Ann Rheum Dis* 50:909, 1991)

One interpretation of the arrangement of the rheumatoid nodule is that it represents an accumulat of immune complexes which contain antibodies and antigen(s), mixed in with fibrin and cell debris, whi attract macrophages from the blood. This forms the palisade of intensely active cells surrounding the necrotic center, ingesting and processing the antigen component, then presenting processed antigenic fragments to adjacent T and B cells in the outer rim of the nodule. In support of this concept has been observation that the $CD4^+$ T cells in the outer rim of the rheumatoid nodule show a marked oligoclonali their antigen receptors compatible with a limited antigenic stimulation (97). This was concluded after measurement of T cell antigen receptor Vβ usage among IL-2 expanded lymphocytes derived from fragı of rheumatoid nodules (See Table 8).

Table 8. OLIGOCLONALITY OF T CELLS EXPANDED FROM RHEUMATOID NODULES BY CULTURE WITH IL-2

	Mab to Sub-Family β-Chain TCR						%
Clone	5A	5B	5C	8	12	6	CD4
TJ1	95	1	1	1	-	-	nd
TJ3	-	-	-	-	-	43	nd
TJ5	-	-	-	-	-	95	nd
BL1	-	-	-	-	95	-	90
BL2	-	-	1	1	3	81	100
BL3	4	1	2	1	79	1	96
BL4	2	1	2	2	75	-	99
BM2	-	-	70	-	-	-	0
BM3	1	1	-	1	1	-	nd
AT1	2	1	4	1	1	68	94
AT2	-	-	-	1	1	11	97
AT4	-	-	-	1	1	47	100
DC1	-	-	50	-	-	-	nd

(DeKeyser, F, et al, *Clin Imunol Immunopathol* 68:29, 1993)

One explanation of the mechanism by which MTX, and to a lesser extent, azathioprine, induces or worsens RA nodules in some patients would be the inhibition of macrophage function and/or proliferation, sufficient to suppress the macrophage component in the synovium, resulting in improvement in the joint inflammation. At the same time, enough macrophage suppression occurs in the rheumatoid nodule that the palisade layer stops eating the central necrotic (?IC) material, and it begins to pile up, not unlike fragments of Ig light chains forming amyloid deposits in some patients with multiple myeloma. The appearance of digital vasculitis (85,87) in some of the methotrexate-treated patients would reinforce the likelihood of IC accumulation as a result of MTX effect on macrophages. There may well be other plausible explanations for this effect of MTX. What function do rheumatoid nodules serve? Are they always detrimental to the patient? Studies of the causes of death in carefully followed groups of patients with RA have given us data regarding cause of death (98) (See Table 9), the physical and serological changes which increase or decrease mortality in RA (99) (Table 10), and the manner in which age and gender influence mortality in RA (99,100).

Table 9. CAUSE OF DEATH IN 898 RA PTS. vs. TOTAL U.S POPULATION

		Proportion	
Category	1985 U.S. Vital Statistics	Observed in RA Pts.	Observed/Expected Ratio
RA	0.001	0.013	12.873
Pneumonia	0.025	0.131	5.307
Other Infections	0.011	0.070	6.213
Leukemia/Lymphoma	0.002	0.019	8.016
Other Cancers	0.277	0.094	0.339
Cardiovascular	0.405	0.402	0.003
CVA	0.064	0.069	1.084
Pulmonary	0.056	0.050	0.902
GI	0.039	0.057	1.466
Injury/Accidents	0.042	0.026	0.606
Renal	0.014	0.012	0.869
All Other	0.064	0.057	0.887

(Modified from Wolfe, E, et al, *Arth Rheum* 37:481, 1994)

Having a positive anti-IgG antibody (rheumatoid factor) in moderate or high titer increases your chan of dying 1.6 to 2.3 times if you have RA, but does not increase your chance of dying if you do not have (99). If you are male and have RA, you are 2.2 times more likely to die than a woman with RA of the s age. This also is true if you are male without RA (1.7 times more likely to die at a given age than a wo without RA of the same age). Things like low dose prednisone use, disease duration, presence of diabete surprisingly do not increase mortality for patients with RA. Surprise! If you have rheumatoid nodules, y are 12% less likely to die at a given age than a patient with RA without nodules. On the other hand, if y had arthritis bad enough to warrant treatment with a disease modifying anti-rheumatic drug (gold, HCQ, SSZ, MTX or D-penicillamine), you had a 27% increased risk of dying. This study (99) represented an analysis of the deaths of 79 patients with RA from the Gila Bend Indian Community in Arizona from 19 through 1989 when compared to mortality risk for the same variables among 779 deaths in patients withc RA during the same time period. There may have been very little use of methotrexate in this group of patients prior to 1989 so that gold therapy was probably the most widely used DMARD in the 1965-89 period. A similar evaluation of mortality over a long period for patients being treated with MTX remain: be done, but conservative estimates suggest that over half of all RA patients in the United States are receiving MTX to control their disease at this time.

Table 10. AGE- AND SEX-ADJUSTED MORTALITY RATE RATIOS IN PIMA INDIANS WITH AND WITHOUT RA (1965-1989)

Risk Factor	RA (79 deaths)	No RA (779 deaths)
Male/Female	2.23	1.74
Diabetes (Yes/No)	1.01	1.39
RF (SCAT (pos/neg)	1.61	1.20
RF (BFT) (pos/neg)	2.29	1.21
RA (Chart +)(yes/no)	1.24	
ACR Criteria (5-6/<5)	1.17	
Nodules (yes/no)	0.88	
Steroid Therapy (yes/no)	1.03	
DEMARD Therapy (yes/no)	1.27	
Duration ((>10 yrs/<10 yrs	1.01	

(Jacobsson, LTH, et al, *Arth Rheum* 36:1045, 1993)

In closing, what can we now say about the etiology of RA? Most investigators agree that RA appear be triggered by an environmental agent in an immunogenetically predisposed person (101). Some form infectious agent remains the most attractive possibility. The indirect evidence presented by Bond and Cleland (101) that prepubertal exposure to a cat (67% of RA patients vs 27% of non-RA control subjects interesting, and perhaps provides a list of potential infectious agents which should be explored if suitable antibodies from RA IC become available.

Acknowledgement. I wish to thank Dr. Wayne L. Hoffman and Mr. Adrien A. Jump for invaluabl assistance in the preparation of this presentation. JDS

BIBLIOGRAPHY

1. Theofilopoulos, AN. 1981. The Raji, conglutinin, and anti-C3 assays for the detection of complement-fixing immune complexes. *Adv Immunol* 30:511-668.

2. Laursen, SB, S Thiel, B Teisner, U Holmskov, Y Wang, RB Sim, and JC Jensenius 1994. Bovine conglutinin binds to an oligosaccharide determinant presented by iC3b, but not by C3, C3b, or C3c. *Immunology* 81:648-654.

3. Roberts-Thomson, PJ. 1982. A reappraisal of the monoclonal rheumatoid factor test for circulating immune complexes:a comparison of two monoclonal rheumatoid factor reagents. *Clin Exp Immunol* 48:52-60.

4. Baumann, MA, and BE Anderson. 1990. An immune complex selective affinity matrix utilizing a synthetic peptide. *J Biol Chem* 265:18414-18422.

5. Tung, KSK, RJ DeHoratius, and RC Williams, Jr. 1981. Study of circulating immune complex size in systemic lupus erythematosus. *Clin Exp Immunol* 43: 615-625.

6. Wener, MH, M Mannik, MM Schwartz, and EJ Lewis. 1987. Relationship between renal pathology and the size of circulating immune complexes in patients with systemic lupus erythematosus. *Medicine* 66:85-97.

7. McDougal, JS, M Hubbard, FC McDuffie, PL Strobel, SJ Smith, N Bass, JA Goldman, S Hartman, G Meyerson, S Miller, R Morales, and CH Wilson,Jr. 1982. Comparison of five assays for immune complexes in the rheumatic diseases. An assessment for their validity in rheumatoid arthritis. *Arth Rheum* 25:1156-1166.

8. Pereira, AB, AN Theofilopoulos, and FJ Dixon. 1980. Detection and partial characterization of circulating immune complexes with F(ab)'2 anti-C3. *J Immunol* 125:763-770.

9. Jacobs, RJ, and M Reichlin. 1983. Generation of low M.W., C3-bearing immunoglobulin in human serum. *J Immunol* 130:2775-2781.

10. Hack, CE, and AJM Belmer. 1986. The IgG detected in the C1q solid-phase assay is not always of immune-complex nature. *Clin Immunol and Immunopathol* 38:120-128.

11. Law, SK, NA Lichtenberg, and RP Levine. 1979. Evidence for an ester linkage between the labile binding site of C3b and receptive surfaces. *J Immunol* 123:1388-1394.

12. Reid, KBM. 1995. The complement system. A major effector mechanism in humoral immunity. *The Immunologist* 3:206-211.

13. Roitt, I, J Brostoff, and D Male. 1996. Complement in *Immunology*, Fourth Edition, Mosby, London, p 13.5.

14. Kozel, TR, MA Wilson, TP Farrell, and SM Levitz. 1989. Activation of C3 and binding to *Aspergillus fumigatus* conidia and hyphae. *Infection and Immunity* 57:3412-3417.

15. Bergamaschini, L, T Santangelo, A Faricciotti, N Ciavarella, PM Mannucci, and A Agostoni. 1996. Study of complement-mediated anaphylaxis in humans. The role of IgG subclasses (IgG1 and/or IgG4) in the complement-activating capacity of immune complexes. *J Immunol* 156:1256-1261.

16. Holers, VM. 1996. Complement in *Clinical Immunology: Principles and Practice*, RR Rich, TA Fleisl BD Schwartz, WT Sherer, and W Strober, eds, Mosby, St. Louis, pp 363-391.

17. Parekh, R , D Isenberg and G Rook. 1989. A comparative analysis of disease-associated changes in galactosylation of serum IgG. *J Autoimmun* 2:101-114.

18. Young, A, N Sumar, K Bodman, S Goyal, H Sinclair, I Roitt, and D Isenberg. 1991. Agalactosyl Ig An aid to differential diagnosis in early synovitis. *Arth Rheum* 34:1425-1429.

19. Axford, JS, N Sumar, A Alavi, DA Isenberg, A Young, KB Bodman, and IM Roitt. 1992. Change normal glycosylation mechanisms in auto immune rheumatic diseases. *J Clin Invest* 89:1021-1031.

20. Bond A, A Alavi, FC Hay, P Youinou, and JS Axford. 1993. Immunoglobulin G glycosylation changes occur in rheumatoid arthritis and primary Sjogren's syndrome but due to different mechanisms. *Rheum* 36:S242 (Abstract).

21. Axford, JS. 1994. Editorial: Glycosylation and rheumatic disease: More than icing on the cake. *J Rheumatol* 21:1791-1795.

22. Hernandez-Pando, R, P Reyes, C Espitia, Y Wang, G Rook and R Mancilla. 1994. Raised agalacto IgG and antimycobacerial humoral immunity in Takayasu's arteritis. *J Rheumatol* 21:1870-1876.

23. Bond, A, MA Kerr, and FC Hay. 1995. Distinct oligosaccharide content of rheumatoid arthritis-derive immune complexes. *Arth Rheum* 38:744-749.

24. Malhotra, R, MR Wormald, PM Rudd, PB Fischer, RA Dwek, and RB Sim. 1995. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannos-binding prot *Nature Medicine* 1:237-243.

25. Miletic VD, and MM Frank. 1995. Complement-immunoglobulin interactions. *Current Opinion in Immunology* 7:41-47.

26. Bond, A, MA Kerr, and FC Hay. 1995. Distinct oligosaccharide content of rheumatoid arthritis-deri immune complexes. *Arth Rheum* 38:744-749.

27. Ezekowitz, RAB. 1995. Agalactosyl IgG and mannose-binding proteins: Biochemical nicety or pathophysiological paradigm? *Nature Medicine* 1:207-208.

28. Nikolova, EB, M Tomana, and MW Russell. 1994. All forms of human IgA antibodies bound to antigen interfere with complement (C3) fixation induced by IgG or by antigen alone. *Scand J Immunol* 39:275-280.

29. Miletic, VD, CG Hester, and MM Frank. 1996. Regulation of complement activity by immunoglobuli Effect of immunoglobulin isotype on C4 uptake on antibody-sensitized sheep erythrocytes and solid phase immune complexes. *J Immunol* 156:749-757.

30. Liszewski, MK, and JP Atkinson. 1993. The complement system. in *Fundamental Immunology*, Third Edition, WE Paul, ed, Raven Press, Ltd, New York, 917-939.

31. Davies, KA, JA Schifferli, and MJ Walport. 1994. Complement deficiency and immune complex dise *Springer Semin Immunopathol* 15: 397-416.

32. Medof, ME, K Iida, C Mold, and V Nussenzweig. 1982. Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. *J Exp Med* 156:1739-1754.

33. Schifferli, JA, YC Ng, and DK Peters. 1986. The role of complement and its receptor in the elimination of immune complexes. *N Eng J Med* 315:488-495.

34. Birmingham, DJ. 1995. Erythrocyte complement receptors. *Critical Reviews in Immunology* 15:133-154.

35. Ross, GD, and ME Medof. 1985. Membrane complement receptors specific for bound fragments of C3. *Advances in Immunology* 37:217-263.

36. Fearon, DT, and R M Locksley. 1996. The instructive role of innate immunity in the acquired immune response. *Science* 272:50-54.

37. Dempsey, PW, MED Allison, S Akkaraju, CC Goodnow, and DT Fearon. 1996. C3d of complement as a molecular adjuvant: Bridging innate and acquired immunity. Science 271:348-350.

38. Molina, H, VM Holers, B Li, Y-F Fang, S Mariathasan, J Goellner, J Strauss-Schoenberger, RW Karr, and DD Chaplin. 1996. Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc Natl Acad Sci USA* 93:3357-3361.

39. Fearon, DT. 1988. Complement, C receptors, and immune complex disease. *Hospital Practice* 23:63-72.

40. Ross, GD, WJ Yount, ML Walport, JB Winfield, CJ Parker, CR Fuller, RP Taylor, BL Myones, and PJ Lachmann. 1985. Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptors) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. *J Immunol* 135:2005-2014.

41. Corvetta, A, G Pomponio, R Bencivenga, MM Luchetti, M Spycher, PJ Spaeth, and G Danieli. 1991. Low number of complement C3b/C4b receptors (CR1) on erythrocytes from patients with essential mixed cryoglobulinemia, systemic lupus erythematosus, and rheumatoid arthritis: Relationship with disease activity, anticardiolipin antibodies, complement activation and therapy. *J Rheumatol* 18:1021-1025.

42. Paccaud, J-P, J-L Carpentier, and JA Schifferli. 1988. Direct evidence for the clustered nature of complement receptors on the erythrocyte membrane. *J Immunol* 141:3889-3894.

43. Ruddy, S, and KF Austen. 1970. The complement system in rheumatoid synovitis. I. An analysis of complement component activities in rheumatoid synovial fluids. *Arth Rheum* 13:713-723.

44. Nydegger, UE, RH Zubler, R Gabay, G Joliat, Ch Karagevrekis, PH Lambert, and PA Miescher. 1977. Circulating complement breakdown products in patients with rheumatoid arthritis. Correlation between plasma C3d, circulating immune complexes, and clinical activity. *J Clin Invest* 59:862-868.

45. Mollnes, TE, T Lea, OJ Mellbye, J Pahle, O Grand, and M Harboe. 1986. Complement activation in rheumatoid arthritis evaluated by C3dg and the terminal complement complex. *Arth Rheum* 29:715-721.

46. Olmez, U, P Garred, TE Mollnes, M Harboe, HB Berntzen, E Munthe. 1991. C3 activation products, C3 containing immune complexes, the terminal complement complex and native C9 in patients with rheumatoid arthritis. *Scand J Rheumatol* 20:183-189.

47. Jepsen, HH, S-E Svehag, L Jarlbaek, and G Baatrup. 1986. Interaction of complement-solubilized immune complexes with CR1 receptors on human erythrocytes. The binding reaction. *Scand J Immunol* 23:65-73.

48. Schifferli, JA, SR Bartolotti, and DK Peters. 1980. Inhibition of immune precipitation by complemer *Clin Exp Immunol* 42:387-394.

49. Czop J, and V Nussenzweig. 1976. Studies on the mechanism of solubilization of immune precipitat serum. *J Exp Med* 143:615-630.

50. Naylor, JF, SA Ward, SE Moore, and JD Smiley. 1979. Decreased complement solubilization of imr complexes in sera containing high titers of rheumatoid factor. *Arth Rheum* 22:642 (Abstract).

51. Balestrieri, G, A Tincani, P Migliorini, C Ferri, R Cattaneo, and S Bombardieri. 1984. Inhibitory eff of IgM rheumatoid factor on immune complex solubilization capacity and inhibition of immune precipita *Arth Rheum* 27:1130-1136.

52. Ng, YC, DK Peters, and MJ Walport. 1988. Monoclonal rheumatoid factor-IgG immune complexes. Poor fixation of opsonic C4 and C3 despite efficient complement activation. *Arth Rheum* 31:99-107.

53. Ross, GD, JD Lambris, JA Cain, and SL Newman. 1982. Generation of three different fragments of bound C3 with purified Factor I or serum. I. Requirements for Factor H vs CR_1 cofactor activity. *J Imm* 129:2051-2059.

54. Jepsen, HH, S-E Svehag, JC Jensenius and RB Sim. 1986. Release of immune complexes bound to erythrocyte complement receptor CR1), with particular reference to the role of Factor I. *Scand J Immuno* 24:205-213.

55. Jepsen, HH, B Teisner, and S-E Svehag. 1990. Zinc ions inhibit Factor I-mediated release of CR1-b immune complexes and degradation of cell-bound complement factors C3b and C4b. *Scand J Immunol* 31:397-403.

56. Vyse, TJ, BJ Morley, I Bartok, EL Theodoridis, KA Davies, ADB Webster and MJ Walport. 1996. The molecular basis of hereditary complement Factor I deficiency. *J Clin Invest* 97:925-933.

57. Vyse, TJ, PJ Spath, KA Davies, BJ Morley, P Philippe, P Athanassiou, CM Giles, and MJ Walport. 1994. Hereditary complement factor I deficiency. *Quart J Med* 87:385-402.

58. Moller Rasmussen, J, B Teisner, HH Jepsen, SE Svehag, F Knudsen, H Kirstein, and M Buhl. 1988. Three cases of factor I deficiency; the effect of treatment with plasma. *Clin Exp Immunol* 74:131-136.

59. Solal-Celigny, P, M Laviolette, J Hebert, PC Atkins, M Sirois, G Brun, G Lehner-Netsch, and JM Delage. 1982. C3b inactivator deficiency with immune complex manifestations. *Clin Exp Immunol* 47:1 205.

60. Yokoyama, I, and F Waxman. 1994. Differential susceptibility of immune complexes to release fr the erythrocyte CR1 receptor by Factor I. *Molecular Immunology* 31:227-240.

61. Hoffman, WL, MS Goldberg, and JD Smiley. 1982. Immunoglobulin G_3 subclass production by rheumatoid synovial tissue cultures. *J Clin Invest* 69:136-144.

62. Shapiro, S, D Kohn, B Miller, and H Gershon. 1994. Erythrocytes from young but not elderly donors can bind and degrade immune complex- and antibody-bound C3 *in vitro. Clin Exp Immunol* 95:181-190.

63. Cornacoff, JB, LA Hebert, WL Smead, ME Van Aman, DJ Birmingham, and FJ Waxman. 1983. Primate erythrocyte immune complex-clearing mechanism. *J Clin Invest* 71:236-245.

64. Taylor, RP, C Horgan, M Hooper, and J Burge. 1985. Dynamics of interaction between complement-fixing antibody/dsDNA immune complexes and erythrocytes. In vitro studies and potential general applications to clinical immune complex testing. *J Clin Invest* 75:102-111.

65. Fischer, D-C, B Opalka, A Hoffmann, W Mayr, and H-D Haubeck. 1996. Limited heterogeneity of rearranged T cell receptors Vα and Vβ transcripts in synovial fluid T cells in early stages of rheumatoid arthritis. *Arth Rheum* 39:454-462.

66. Mima, T, Y Saeki, S Oshima, N Nishimoto, M Matsushita, M Shimizu, Y Kobayashi, T Nomura, and T Kishimoto. 1995. Transfer of rheumatoid arthritis into severe combined immunodeficient mice. The pathogenetic implications of T cell populations oligoclonally expanding in the rheumatoid joints. *J Clin Invest* 96:1746-1758.

67. Ikeda, Y, K Masuko, Y Nakai, T Kato, T Hasanuma, S-I Yoshino, Y Mizushima, K Nishioka, and K Yamamoto. 1996. High frequencies of identical T cell clonotypes in synovial tissues of rheumatoid arthritis patients suggest the occurrence of common antigen-driven immune responses. *Arth Rheum* 39:446-453.

68. Cohen, SBA, PD Katsikis, C-Q Chu, H Thomssen, LMC Webb, RN Maini, M Londei, and M Feldmann. 1995. High level of interleukin-10 production by the activated T cell population within the rheumatoid synovial membrane. *Arth Rheum* 38:946-952.

69. Smiley, JD, C Sachs, and M Ziff. 1968. In vitro synthesis of immunoglobulin by rheumatoid synovial membrane. *J Clin Invest* 47:624-632.

70. Hoffman, WL, AA Jump, and JD Smiley. 1990. Synthesis of specific IgG idiotypes by rheumatoid synovium. *Arth Rheum* 33:1196-1204.

71. Bridges, SL Jr, SK Lee, ML Johnson, JC Lavelle, PG Fowler, WJ Koopman, and HW Schroeder, Jr. 1995. Somatic mutation and CDR3 lengths of immunoglobulin k light chains expressed in patients with rheumatoid arthritis and normal individuals. *J Clin Invest* 96:831-841.

72. Tamerius, JD, MK Pangburn, and HJ Muller-Eberhard. 1985. Detection of a neoantigen on human C3bi and C3d by monoclonal antibody. *J Immunol* 135:2015-2019.

73. Aguado, MT, JD Lambris, GC Tsokos, R Burger, D Bitter-Suermann, JD Tamerius, FJ Dixon, and AN Theofilopoulos. 1985. Monoclonal antibodies against complement 3 neoantigens for detection of immune complexes and complement activation. Relationship between immune complex levels, state of C3, and numbers of receptors for C3b. *J Clin Invest* 76:1418-1426.

74. Kanayama, Y, Y Kurata, R McMillan, JD Tamerius, N Negoro, and JG Curd. 1986. Direct quantitation of activated C3 in human plasma with monoclonal anti-iC3b-C3d-neoantigen. *J Immunological Methods* 88:33-36.

75. Garred, P, TE Mollnes, T Lea, and PJ Lachmann. 1989. Enzyme immunoassay detection of circulat immune complexes by monoclonal antibodies to C3 neoepitopes with special reference to IgG concentrat and to interfering anti-immunoglobulin antibodies. *J Immunological Methods* 117:59-66.

76. Bedwell, AE, CJ Elson, SD Carter, PA Dieppe, CW Hutton, and R Czudek. 1987. Isolation and analysis of complement activating aggregates from synovial fluid of patients with rheumatoid arthritis us: monoclonal anti-C3d antibodies. *Ann Rheumatic Diseases* 46:55-64.

77. Amlot, PL. 1989. Neoepitopes expressed by immune complexes. *Complement Inflamm* 6:236-248.

78. Jarvis, JN, MM Diebold, MK Chadwell, M Iobidze, and HT Moore. 1995. Composition and biolo behavior of immune complexes isolated from synovial fluid of patients with juvenile rheumatoid arthritis (JRA). *Clin Exp Immunol* 100:514-518.

79. Hoffman, WL, A Camann, and AA Jump. 1996. Complement-mediated clearance of immune complexes (IC) in the blood and synovial fluid (SF) of rheumatoid arthritis (RA) patients. *Arth Rheum* 39:Abstract (submitted May 31, 1996, to American College of Rheumatology, Orlando, FL meeting).

80. Morales-Piga, A, A Elena-Ibanez, AC Zea-Mendoza, A Rocamora-Ripoll and J Beltran-Gutierrez. 19 Rheumatoid nodulosis: Report of a case with evidence of intraosseus rheumatoid granuloma. *Arth Rheum* 29:1278-1283.

81. Pearson, ME, M Kosco, W Huffer, W Winter, JA Engelbrecht, and JC Steigerwald. 1987. Rheumatc nodules of the spine: Case report and review of the literature. *Arth Rheum* 30:709-713.

82. Kremer, JM, and JK Lee. 1986. The safety and efficacy of the use of methotrexate in long-term ther for rheumatoid arthritis. *Arth Rheum* 29:822-831.

83. Weinblatt, ME, DE Trentham, PA Fraser, DE Holdsworth, KR Falchuk, BN Weissman, and JS Cobl 1988. Long-term prospective trial of low-dose methotrexate in rheumatoid arthritis. *Arth Rheum* 31:16 175.

84. Jeurissen, MEC, AMTh Boerbooms, and LBA van de Putte. 1989. Eruption of nodulosis and vascu during methotrexate therapy for rheumatoid arthritis. *Clin Rheumatol* 8:417-419.

85. Segal, R, D Caspi, M Tishler, B Fishel and M Yaron. 1988. Accelerated nodulosis and vasculitis during methotrexate therapy for rheumatoid arthritis. *Arth Rheum* 31:1182-1185.

86. Alarcon, GS, WJ Koopman and MJ McCarty. 1993. Nonperipheral accelerated nodulosis in a methotrexate-treated rheumatoid arthritis patient. *Arth Rheum* 36:132-133.

87. Combe, B, C Didry, M Gutierrez, JM Anaya, and J Sany. 1993. Accelerated nodulosis and systen manifestations during methotrexate therapy for rheumatoid arthritis. *Eur J Med* 2:153-156.

88. Langevitz, P, L Maguire, and M Urowitz. 1991. Accelerated nodulosis during azathiaprine therapy *Arth Rheum* 34:123-124.

89. McCarty, D. 1991. Complete reversal of rheumatoid nodulosis. *J Rheumatol* 18:736-737.

90. O'Dell, JR, CE Haire, N Erikson, W Drymalski, W Palmer, PJ Eckhoff, V Garwood, P Maloley, LW Klassen, S Wees, H Klein, and GF Moore. 1996. Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. *N Eng J Med* 334:1287-1291.

91. Bautista, BB, E Boyce, M Koronkowski, and HR Schumacher, Jr. 1992. Effects of second line drugs on progression or regression of subcutaneous rheumatoid nodules. *Arth Rheum* 35:R38 (Abstract).

92. Palmer, DG, N Hogg, J Highton, PA Hessian and I Dehholm. 1987. Macrophage migration and maturation within rheumatoid nodules. *Arth Rheum* 30:729-736.

93. Miyasaka, N, K Sato, K Yamamoto, M Goto, and K Nishioka. 1989. Immunological and immunohistochemical analysis of rheumatoid nodules. *Ann Rheum Diseases* 48:220-226.

94. Ziff, M, T Kantor, E Bien, A Smith. 1953. Studies on the composition of the fibrinoid material of the subcutaneous nodule of rheumatoid arthritis. *J Clin Invest* 32:1253-1259.

95. Ziff, M. 1990. The rheumatoid nodule. *Arth Rheum* 33:761-767.

96. Mellbye, OJ, O Forre, TE Mollnes, L Kvarnes. 1991. Imunopathology of subcutaneous rheumatoid nodules. *Ann Rheum Diseases* 50:909-912.

97. De Keyser, F, G Verbruggen, EM Veys, C Cuvelier, A-M Malfait, D Benoit, D Elewaut, J Vermeersch, and A Heirwegh. 1993. T cell receptor Vβ_usage in rheumatoid nodules: Marked oligoclonality among IL-2 expanded lymphocytes. *Clin Immunol Immunopathol* 68:29-34.

98. Jacobsson, LTH, WC Knowler, S Pillemer, RL Hanson, DJ Pettitt, RG Nelson, A del Puente, DR McCance, M-A Charles, and PH Bennett. 1993. Rheumatoid arthritis and mortality. A logitudinal study in Pima Indians. *Arth Rheum* 36:1045-1053.

99. Wolfe, F, DM Mitchell, JT Sibley, JF Fries, DA Bloch, CA Williams, PW Spitz, M Haga, SM Kleinheksel, and MA Cathey. 1994. The mortality of rheumatoid arthritis. *Arth Rheum* 37:481-494.

100. Anderson, ST. 1996. Mortality in rheumatoid arthritis: Do age and gender make a difference? *Semin Arthritis Rheum* 25:291-296.

101. Bond, C and LG Cleland. 1996. Rheumatoid arthritis: Are pets implicated in its etiology? *Semin Arth*ritis *Rheum* 25:308-317.