Detection of $\alpha_2$-Macroglobulin-Associated Proteases in the Plasma of Patients with Rheumatoid Arthritis

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In previous work a polyclonal B cell activator has been detected in the serum of patients with rheumatoid arthritis (RA). This activator is associated with $\alpha_2$-macroglobulin ($\alpha_2$M) and its activity is blocked by low-M$_r$ trypsin inhibitors, which suggests that it may be a protease-$\alpha_2$M complex. Here we determined the possibility of developing a routine clinical chemistry test for detection of this complex in patients' blood. We measured with chromatographic substrates the total proteolytic activity of citrated plasma and of the $\alpha_2$M immunoabsorbed from plasma. Low-M$_r$ substrates containing Arg were degraded much better by plasma from RA patients than by plasma from patients with other arthritides. Low-M$_r$ substrates containing Leu, Lys, or Gly or the large-M$_r$ substrate Azocoll were not degraded by RA patients' plasma. $\alpha_2$M from RA patients' plasma attached to a solid-phase immunoabsorbent degraded an Arg-containing tripeptide much better than did the $\alpha_2$M from normal donors, from patients with systemic lupus erythematosus, or from those with joint inflammation of other, "non-autoimmune" origin. Although the enzyme associated with $\alpha_2$M in the plasma from RA patients appeared to be similar to trypsin, the differences in optimal pH, cation concentration, degradation of Lys-containing substrates, and biological activity suggest otherwise. We speculate that the $\alpha_2$M-protease complexes are generated in the immune system and contribute to the inflammatory and autoimmune phenomena in RA.

**Additional Keyphrases:** studies with immunoabsorbent-linked protein • trypsin-like enzyme • autoimmune disorders • B cells • synthetic substrates • systemic lupus erythematosus • lymphokines

Polyclonal gammopathy and production of autoantibodies are frequent findings in patients with rheumatoid arthritis (RA), systemic lupus erythematosus, and other autoimmune diseases.8 In previous work we detected a factor in patients' blood that caused polyclonal B cell activation (1–4). This factor is associated with $\alpha_2$-macroglobulin ($\alpha_2$M) and is blocked by low-M$_r$ protease inhibitors, which suggests that it may be a protease (4).

Other experiments have suggested that $\alpha_2$M-protease complexes may play a role in the polyclonal B cell activation and in the inflammatory process in RA. These types of complexes have been found not only in the supernates of high-density lymphoid cell cultures (5) but also, in large amounts, in the sera of rabbits inoculated intravenously with allogeneic lymph node cells (6). Moreover, the $\alpha_2$M from RA patients' serum (2, 3) or from the supernate of the lymphoblastoid cell lines RPMI-1788 (McIntire and Papermaster, personal communication) produces inflammation when injected intradermally in guinea pigs. Finally, repeated intra-articular injections of $\alpha_2$M–trypsin complexes in rabbits cause an inflammatory process resembling that seen in RA (7).

On the basis of these observations we investigated the possibility of using a chromatographic substrate to detect in RA patients any increased protease activity in plasma or any increase in the proportion of $\alpha_2$M molecules in plasmas that contain proteases. The results presented here strongly suggest that RA patients had a greater concentration of protease activity and of $\alpha_2$M–protease complexes than did normal donors or patients with other arthritides.

**Materials and Methods**

**Patients and Sample Collection**

We collected blood from patients with classical "seropositive" RA, systemic lupus erythematosus, or other arthritides (i.e., psoriatic arthritis, gout, osteoarthritis) and from normal donors. The blood was collected in Vacutainer Tubes containing no anticoagulant or either sodium citrate (3.8 mg/mL of blood), EDTA (2 mg/mL of blood), or heparin (0.2 mg/mL of blood) (Becton-Dickinson and Co., Rutherford, NJ). The plasma, collected by centrifugation for 10 min at 3000 x g, was either tested immediately or frozen and stored at −25 °C. To collect serum, we incubated the whole blood at 37 °C for 1 h, centrifuged, then froze the serum and stored it at −25 °C. Serum was also obtained from plasma containing sodium citrate by adding CaCl$_2$ to a final concentration of 0.2 mol/L, and incubating at 37 °C for 1 h.

**Reagents**

**Enzymes and substrates.** We prepared a stock solution of bovine trypsin (EC 3.4.21.4; from Sigma Chemical Co., St. Louis, MO), 1 mg/mL in 2 mmol/L HCl, and stored it in aliquots at −25 °C. We used the following protease substrates. Carbobenzyoxy-Val-Gly-Arg-p-nitroanilide (Chromozym Try; Boehringer Mannheim, Indianapolis, IN) was dissolved in ethanol (20 mg/mL) and then diluted to 1 mg/mL with distilled water; L-Leu-p-nitroanilide (LPN; Boehringer Mannheim), 1 mg/mL, was prepared similarly. For separate solutions of benzoyl-Arg-p-nitroanilide
(BAPNA; Boehringer Mannheim) and N-α-benzyol-Arg-β-naphthylamide (BANA; Sigma) we dissolved 43.5 mg of either in 1 ml of dimethyl sulfoxide and diluted 100-fold with distilled water. N-α-p-Toluene sulfonyl-l-Arg-methyl ester (TAME; Sigma) was dissolved directly in distilled water to 1 mg/ml. We suspended 50 mg of Azocoll (Calbiochem Behring, LaJolla, CA) in 5 ml of phosphate buffer, 0.1 mol/L, pH 7.0. All substrates were stored in the dark at 4°C.

Protease inhibitors. The stock solution of phenylmethylsulfon fluoride (Sigma) was 0.1 mol/L in ethanol. The stock solution of soybean trypsin inhibitor (Sigma), 20 mg/ml of phosphate-buffered saline (PBS) at pH 7.2, was dialyzed overnight against PBS in a dialysis tubing with a M<sub>c</sub> cutoff of 10 000 (Spectra Por; Pierce, Rockford, IL). The aprotinin (Boehringer Mannheim) stock solution contained 10 000 kallikrein inhibitory units per milliliter of PBS. All inhibitor solutions were stored at -25°C.

Procedures

Determination of protease activity in plasma or serum. After diluting the sample of plasma or serum fourfold with PBS, we mixed 0.1 ml of this with 0.1 ml of Chromozym Try, LPN, TAME, or Azocoll solutions or with 0.2 ml of BANA or BAPNA solutions. For the reaction mixtures containing Chromozym Try, LPN, TAME, or BAPNA we adjusted the volume to 0.6 ml with Tris·HCl buffer (50 mol each of Tris and NaCl per liter, pH 8.2). For BANA and Azocoll we adjusted the volume with phosphate buffer (0.1 mol/L, pH 7.0). The controls were constituted as follows: (a) the plasma or serum was replaced by the buffer; (b) the chromogenic substrate was replaced by buffer; or (c) the plasma or serum was replaced by trypsin solution (stock solution diluted 300-fold). Each sample was processed in triplicate.

All mixtures except those containing BANA were incubated at 20°C for 2 h, then diluted to 1.2 ml with H<sub>2</sub>O, after which we measured their absorbances with a Beckman spectrophotometer (Model 25) and semimicro cuvettes. The absorbance for TAME was determined at 247 nm, for Azocoll at 520 nm, and for all the other substrates, which release p-nitroaniline, at 380 nm.

When BANA was used, we incubated the mixture at 20°C for 1 h, then added 0.1 ml of a freshly prepared solution of Fast Garnet Salt (Sigma), 3 mg/ml. After another hour of incubation at 20°C we stopped the reaction by adding 0.5 ml of acetate buffer (1 mol/L, pH 4.2, containing 100 g of Tween 20 per liter), according to Hopeu and Gienner (8) and determined the absorbance at 525 nm.

Preparation of anti-α<sub>2</sub>M-coated beads. We coated Affigel 10 beads (Biorad, Richmond, CA) with goat anti-human α<sub>2</sub>M antibodies (Cappel Lab Industries, Westchester, PA). For controls, we also coated beads with normal goat IgG (Cappel Lab Industries) and used other beads uncoated. To 1 ml of Affigel 10 beads we added 20 mg of antibodies in 1 ml of NaHCO<sub>3</sub> buffer (0.1 mol/L, pH 8.5). After gentle rocking at 4°C for 4 h we washed the beads with more NaHCO<sub>3</sub> buffer. The remaining active esters on the beads were blocked by incubation for 1 h at 4°C with 0.2 mol/L glycine buffered with 0.1 mol/L Tris (pH 8.2). The uncoated beads were also treated with glycine-Tris buffer. We then washed all the beads with borate saline buffer (0.16 mol/L, pH 8.2) and stored them at 4°C in the same buffer.

Assay of α<sub>2</sub>M-associated proteolytic activity. We mixed 0.1 ml of plasma (diluted fourfold with PBS) with another 0.1 ml of PBS and 50 μL of anti-α<sub>2</sub>M-coated beads washed in PBS. As a control, we mixed the diluted plasma with normal goat IgG-coated beads or with uncoated beads. Each sample was prepared in triplicate.

After incubating the mixtures at 37°C on a rocking platform (Lab Industries, Berkeley, CA) for 2 h, we washed the beads three times with PBS by centrifugation at 750 × g for 5 min. After the final wash each tube contained the beads in 0.2 ml of PBS; to each we added 0.1 ml of Chromozym Try and adjusted the final volume to 0.6 ml with Tris·HCl buffer (pH 8.2). We incubated the mixture again for 2 h at 20°C with continuous rocking, then added 0.6 ml of distilled water and centrifuged the tubes at 750 × g for 10 min before measuring the absorbance of each sample at 380 nm.

Inhibition of proteases. To inhibit proteases in whole plasma, we incubated equal volumes of fourfold-diluted plasma and inhibitor or solvent at 37°C for 1 h. After this step, we tested for protease activity as described above.

To inhibit proteases associated with α<sub>2</sub>M, we first incubated the plasma with the beads coated with anti-α<sub>2</sub>M antibodies, as described above. The beads coated with α<sub>2</sub>M from patients' plasma in PBS were mixed with an equal volume of inhibitor solution and incubated at 37°C for 1 h with continuous rocking. The degradation of the substrate was determined as described above.

Results

Collection and Storage of Plasma

Our previous work suggested that a trypsin-like protease associated with α<sub>2</sub>M was present at higher activities in the plasma of patients with RA than in normal individuals (4). Therefore, we attempted first to use Chromozym Try (a trypsin substrate) to measure the total proteolytic activity in plasma of three normal donors and one patient with RA. The lowest value of proteolytic activity toward Chromozym Try was seen in plasma containing citrate and the highest was in serum (Table 1). EDTA-containing plasma gave values slightly higher than those obtained with citrate (data not shown). The differences between the enzyme activity of the plasma of the three normal donors and that of the RA patient was clearly higher with citrated plasma than with heparinized plasma. Conversion of plasma to serum caused a substantial increase in the enzyme activity and obscured any difference between normal donors and the patient. This increase strongly suggested that in the serum, and very likely in the plasma containing heparin, most of the activity was due to enzymes of coagulation.

To determine whether the enzyme activity of citrated plasma changes with time, we incubated the samples at room temperature for various periods before testing for degradation of Chromozym Try. For up to 2 h of storage at room temperature the proteolytic activity was unchanged (Figure 1), but increased significantly at 3 h and 4 h. However, adding soybean trypsin inhibitor at time 0 kept the activity unchained, even after 4 h of incubation.

To determine whether citrated plasma could be stored

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<th>Table 1. Effect of Anticoagulants on Enzyme Activity of Whole Plasma and Serum</th>
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*Absorbance (mean ± SE) of p-nitroaniline released from Chromozym Try incubated with plasma or serum for 2 h at 20°C. n = 3 each.
Selection of Substrate and Optimization of Assay

In previous work we had shown that $\alpha_2$M from the serum of patients with RA (4) or from the serum of rabbits inoculated intravenously with allogenic lymphocytes (6) degrade TAME. However, the values obtained were relatively low. To determine which substrate yields the highest values with plasma from RA patients, as compared with normal plasma, and to show the specificity of the enzyme for substrate, we performed the following experiment. Plasmas from five normal donors and five RA patients were tested for the ability to degrade several low-$M_r$ substrates containing arginine, lysine, leucine, or glycine. In addition, we tested Azocoll, a particulate substrate for trypsin and therefore inaccessible to $\alpha_2$M-associated enzymes.

The highest overall values of degradation were obtained with Chromozym Try substrate followed in order by TAME, BANA, and BAPNA (Figure 2). All four substrates showed clear differences in the amount of proteolytic activity between normal and RA patients’ plasma. Moreover, patient E, whose plasma degraded the most Chromozym Try, also had the greatest or near-greatest effect on the other three substrates, and patient B, with the least effect on Chromozym Try, also had the least effect on the other three substrates. Thus, all four substrates containing arginine were apparently capable of indicating differences between the proteolytic activity of patients’ plasma and normal plasma. All these substrates were also degraded by trypsin. In fact, the high values of enzyme activity in patients’ plasma seemed equivalent to the enzyme activity of about $15 \mu g$ of trypsin per milliliter (Figure 2).

The substrates containing other amino acids such as leucine (Figure 2), glycine, or lysine (data not shown) were not significantly degraded and thus showed no differences between plasma from RA patients and that from normals. Azocoll was degraded by trypsin but not by normal plasma or RA patients’ plasma; presumably, the plasma enzymes did not have access to this large substrate, probably because of their association with $\alpha_2$M (see below).

The optimal pH for trypsin activity is 9.0. Thus, to determine whether the protease in RA patients’ plasma and serum had the same pH of optimal enzyme activity, we incubated fourfold-diluted plasma or eightfold-diluted serum with Chromozym Try at various pH values. Plasma was incubated in the Tris-saline buffer; serum and trypsin solutions were incubated in the same buffer but with 50 mmol of CaCl$_2$ added per liter. The optimal pH of degradation of Chromozym Try was 9 for trypsin and between 8 and 8.5 for the protease in the patients’ plasma and serum (Figure 3). Thus, we performed all other experiments at pH 8.0.
8.2. The enzyme activity measured in other buffers such as phosphate and borate was generally lower than in Tris buffer.

To determine which cation best facilitates the expression of enzyme activity of plasma or serum from RA patients and to determine whether Ca²⁺ affects the expression of this activity, we performed the following experiment. The proteolytic activity of plasma or serum collected from RA patients was determined with Chromozym Try as a substrate in the presence of the following cations, 50 mmol/L in Tris buffer: Na⁺ (Na₂C₆H₅O₇ · 2H₂O), Ca²⁺ (CaCl₂), K⁺ (KCl), or Fe³⁺ ((C₆H₅FeO₇ · H₂O). Trypsin solution was used as a control. The addition of CaCl₂ increased the proteolytic activity of serum, trypsin, and plasma by about 20% (clotting did not occur, probably because of the use of diluted plasma). The addition of Na⁺, either as NaCl or as Na citrate, also increased the proteolytic activity of plasma. The ferric citrate reduced the proteolytic activity of all three enzyme-containing solutions (Table 2).

To determine the optimal molarity of Na in the reaction mixture, we incubated citrated plasma from RA patients with Chromozym Try in Tris buffer in the presence of increasing concentrations of NaCl. As a control we also added increasing concentrations of CaCl₂ and examined trypsin and serum samples in addition to plasma. The proteolytic activity of plasma was optimal at in the presence of 50 mmol of NaCl per liter (Figure 4). CaCl₂ at low concentrations increased the proteolytic activity of plasma but reduced it at high concentrations; however, these high concentrations of CaCl₂ did not reduce the activity of trypsin.

To determine the optimal time and temperature of incubation on the degradation of Chromozym Try by patients' plasma, we incubated the reaction mixtures in disposable cuvettes containing patients' plasma or normal plasma at room temperature or at 37 °C in a waterbath and measured A₃₅₀ at 30-min intervals. The maximum difference in p-nitroaniline release between normal and RA plasma was observed after 150-min incubation. There was practically no difference between the values obtained at 37 °C and 20 °C (Figure 5). This lack of difference may be due to the excess of substrate and its interaction with other plasma proteins, or to insufficient substrate. However, there was a large difference between results at the two temperatures when we used trypsin solution instead of plasma (data not shown).

Under the conditions described above we tested samples in no particular order from four groups of patients: normal donors, patients with other arthritides (osteoarthritis, five), psoriatic arthritis (two), and gout (three), 10 patients with RA, and 10 with systemic lupus erythematosus. The proteolytic activity for Chromozym Try was significantly greater in patients with RA than in all other groups (Figure 6).

Association of Proteolytic Activity in Patient's Plasma with α₂M

As is well known, proteases associated with α₂M can degrade low-Mr substrates and are inhibited by low-Mr inhibitors but do not interact with large substrates or inhibitors. Thus, we determined, in plasma of the same patients as in Figure 6, the degradation of Chromozym Try by α₂M attached to an immunoabsorbent. In a first series of experiments we determined the conditions of saturation of the immunoabsorbent with plasma α₂M (data not shown). The amount degraded by α₂M from plasma of patients with RA was much greater than by α₂M from normal donors or from patients with lupus or other arthritides. The values obtained with plasma from the other patients did not differ from those obtained with normal plasma.

To determine that indeed the association of enzyme activity was with α₂M and not with some other enzyme that reacted with the goat anti-α₂M antibodies, we performed a competition experiment. Some agarose beads coated with goat anti-α₂M antibody were first treated with purified α₂M from normal serum before use in the assays; this normal α₂M had been pretreated with methylamine to prevent any additional binding of proteases (9). Control beads in this experiment were treated with normal human IgG. The ability of the solid phase to attach the protease from RA patients' plasma was completely blocked by pretreatment with normal α₂M but was not affected by human IgG. For

![Table 2. Effect of Cations on the Proteolytic Activity of Plasma or Serum from RA Patients or of Trypsin](https://example.com/table2.png)
example, for a background degradation value of 0.190 (A380) the beads pre-treated with saline or IgG had values of 0.420 and 0.426, respectively; the beads pre-treated with α2M had a value of 0.191, i.e., practically equal to the background.

In addition we determined the amount of Chromozym Try that was degraded by the solid-phase immunoabsorbent treated with patients’ plasma in the presence of protease inhibitors of high Mw [soybean trypsin inhibitor (20 μg/mL)] and low Mf [phenylmethylsulfonyl fluoride (1 mmol/L) and aprotinin (5000 kallikrein inhibitory units/mL)]. For controls, we prepared trypsin–α2M complexes and used them instead of patients’ plasma. The two low-Mf inhibitors blocked completely the proteolytic activity of both trypsin–α2M complexes and patients’ plasma; soybean trypsin inhibitor inhibited only 18% (range 7% to 40% in six patients) of the activity of patients’ plasma and 8% of that of trypsin–α2M complexes. The slight but significant inhibition by soybean trypsin inhibitor and the complete inhibition by phenylmethylsulfonyl fluoride or aprotinin are well-known characteristics of α2M–trypsin complexes (10). Thus, the increase in proteolytic activity in RA patients’ plasma appears to be due to a trypsin-like enzyme associated with α2M.

Discussion

We showed here that the proteolytic activity of plasma from patients with RA is higher than that of normal donors or of patients with other "non-autoimmune" arthropides or with systemic lupus erythematosus, and we have determined the optimal conditions of pH, time of incubation, substrates, etc. for detecting these differences. This activity can be measured either in whole citrated plasma or on a solid-phase immunoabsorbent containing anti-α2M antibodies.

The higher than normal degradation of Chromozym Try and of other substrates by plasma from patients with RA appears to be due a trypsin-like enzyme, as indicated by the necessity for the presence of arginine at the point of attack. However, there are three differences. First, the optimal pH for patients’ plasma is lower than that for trypsin. Second, a substrate containing lysine was degraded by trypsin but not by RA patients’ plasma. Thirdly, Ca2+ exceeding 50 mmol/L does not affect trypsin but inhibits the proteolytic activity of patient’s plasma. However, the exact nature of the enzyme or enzymes responsible for the degradation of Chromozym Try by plasma from RA patients remains to be determined, particularly because some enzymes of coagulation can also degrade this substrate.

The enzyme activity primarily was associated with α2M, although some free enzymes probably also contributed to the degradation of the substrates. These free enzymes may account for the increase in differences between RA patients’ plasma and controls when we determined the activity associated only with anti-α2M bound to immunoabsorbent (Figure 6). The primary association with α2M was demonstrated on the basis of immunoabsorption with anti-α2M antibody and on the evidence that normal α2M can effectively compete with the enzyme activity attached to the solid phase. Also, when anti-Ig light-chain antibodies were used instead of anti-α2M, there was practically no increase in protease activity (data not shown). Moreover, soybean trypsin inhibitor was relatively inefficient in blocking the proteolytic activity, but low-Mf inhibitors, aprotinin and phenylmethylsulfonyl fluoride, completely blocked this activity. The proteolytic activity associated with α2M represents only the proportion of α2M molecules that carries proteases, the immunoabsorbent having been used under conditions of saturation.

The origin of the increased enzyme activity in plasma from RA patients has not yet been determined. It may be generated by the coagulation system, because some of the enzymes involved in coagulation such as thrombin or plasmin (11) can also degrade Chromozym Try and other substrates containing arginine; moreover, these coagulation enzymes can bind to α2M (12). Several facts make a primary role for coagulation enzymes unlikely. First, in the presence of citrate the enzyme activity undergoes practically no increase for 2 h, which suggests that the coagulation enzymes are kept under control for the period of time of the assay. Second, the α2M from normal human serum degraded TAME much less than the α2M from the serum of RA patients (4); third, we have observed similar differences between normal rabbit αM (the rabbits have two α-macroglobulins, a1 and a2) and αM from the serum of rabbits inoculated with allogeneic lymphoid cells (6). Thus, it appears that the proteolytic activity associated with α2M may be greater in RA patients than in normals even when the coagulation system is activated. This aspect requires further investigation.

Complement system proteases are also an unlikely source of this activity because they do not associate with α2M (13). Moreover, the α2M from patients with systemic lupus erythematosus had less activity than that from RA patients, although it is well known that complement is more activated in the blood of systemic lupus erythematosus patients than in the blood of RA patients.

Two other possibilities remain to be investigated. One is the production of enzymes by the inflammatory cells in the synovium; these enzymes could be produced in large amounts and could be released in the blood and become associated with α2M. This hypothesis would not explain, however, the experiments in rabbits inoculated with allogeneic cells (6). Also, we have seen patients with psoriatic
arthritis with substantial synovitis but normal concentrations of $\alpha_2M$–protease complexes, and patients with joints completely cleared by use of aspirin, who continued to have high activities of protease in the blood (unpublished observations). Thus, as has to be determined in longitudinal studies, the measurement of $\alpha_2M$–protease complexes may give information regarding the underlying chronic inflammatory process. So far no clinical laboratory test can give such indication.

The other possibility, which we favor at this time, is that the protease–$\alpha_2M$ complexes are generated in the immune system. In previous work we have shown that close contacts between rabbit lymphocytes in vitro leads to the production of a factor, recovered in the $\alpha_2M$ fraction, that activates B cells polyclonally. The activity of this factor is blocked by aprotinin (5) but not by soybean trypsin inhibitor (3). The same type of activity was observed with $\alpha_2M$ from the serum of rabbits immunologically activated by inoculation of allogeneic lymphocytes (6). Moreover, $\alpha_2M$ purified from the serum of patients with RA also activates B cells polyclonally and this activity is blocked by aprotinin (3). Taken together, these data suggest that $\alpha_2M$–protease complexes may be generated by the immune system. In fact, active secretion of $\alpha_2M$ by lymphocytes (14) or by macrophages (15) has been demonstrated.

From the above data we speculate that the protease activity detected in whole plasma or in $\alpha_2M$ attached to an immunoabsorbent represents the enzyme activity of a lymphokine. If this is indeed demonstrated in future studies, it may lead to routine measurement of this lymphokine in patients’ blood.

The cause of inflammation in the synovium of RA patients and of the progressively destructive process is still unknown. The factors of acute inflammation—immune complexes, prostaglandins, and enzymes of polymorphonuclear cells—may contribute to a certain extent. However, although the nonsteroidal anti-inflammatory drugs can reduce this inflammation substantially, they do not alter the course of this process in RA. Some other, as yet undetermined inflammatory agents or chronic-phase reactants may be responsible. The $\alpha_2M$–protease complexes along with other lymphokines may be candidates for such pathogenic factors.

The role of $\alpha_2M$–protease complexes in the inflammatory process in RA has some experimental basis. For example, intra-articular inoculation of normal $\alpha_2M$ in rabbits has no effect; but inoculation of $\alpha_2M$–trypsin complexes causes an inflammatory synovitis similar to that seen in RA patients (7). Also, $\alpha_2M$ from patient’s serum causes inflammation in guinea pigs 6–8 h after intradermal inoculation (2). These complexes also have other effects: they activate macrophages to produce neutral proteases (16); are incorporated faster than native $\alpha_2M$ by cells of the reticuloendothelial system (14) and by fibroblasts (9); are suppressive for T cell responses (18); and are chemotactic for neutrophils (19).

On the basis of previous work on the generation of $\alpha_2M$–protease complexes and on their biological properties (see ref. 3), we propose the following hypothetical model for the pathogenesis of RA. Because of the action of infectious or metabolic factors on a genetically predisposed organism, the normal homing of lymphocytes is altered. Prolonged cell–cell contacts between B and T cells results in their mutual activation and production of lymphokines, including $\alpha_2M$–protease complexes. These complexes are cleared from the blood up to the point of saturation of the system, when their concentrations increase and cause inflammation in target organs, including the joints. These complexes also activate B cells polyclonally, producing polyclonal gammopathy and development of autoantibodies and immune complexes.

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References