SUPPRESSION OF LEUKOCYTE CHEMOTAXIS BY HUMAN IgA MYELOMA COMPONENTS*

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Recent studies by Lawrence et al. (1) have provided evidence for polymorphonuclear leukocyte receptors capable of binding IgA. In addition, our previous studies have described the presence of circulating IgA-associated inhibitors of leukocyte chemotaxis in patients with liver disease (2). Elevation in concentration of serum IgA is a common occurrence in patients with various forms of subacute or chronic liver disease (3, 4); moreover, our studies have suggested some parallel between degrees of serum chemotactic inhibitory activity (CIA)[1] and quantitative estimations of serum IgA. The present study was aimed at examining the influence of serum and isolated M components from patients with IgA myeloma on leukocyte chemotaxis in vitro. It would appear that IgA may in some way directly affect leukocyte chemotaxis possibly by steric hindrance of chemotactic receptors or by other cell-associated modulating influences.

Materials and Methods

Cell Preparation, Chemotaxis, and Random Mobility. Human polymorphonuclear leukocytes (PMNs) were prepared from peripheral blood of healthy adult donors. Blood was mixed with heparin, 10 U/ml, to prevent coagulation. Neutrophil preparations were obtained as previously described (2, 5–7) by Plasmagel sedimentation of erythrocytes and resuspension of leukocytes to a concentration of 7×10^6 PMNs/ml in Hanks' balanced salt solution (HBSS). In some experiments mononuclear cells were removed from these preparations by Ficoll-Hypaque centrifugation (8). Cell suspensions obtained by this combined technique contained greater than 95% PMNs. Monocytes were prepared by Ficoll-Hypaque centrifugation of whole blood (8), and the band of mononuclear cells was collected, washed, and adjusted to a cell concentration of 5×10^6 cells per ml with HBSS. This preparation contained from 11 to 40% monocytes as determined by peroxidase staining (9).

Studies of PMN chemotaxis were performed using a modified Boyden technique (2, 5–7). Chemotaxis was expressed as a chemotactic index representing the average number of cells per high power field (400 ×) which had migrated to the distal side of the membrane. Monocyte chemotaxis was measured in a similar type of chamber with a 5 μm pore size Nuclepore membrane (Nuclepore Corp., Pleasanton, Calif.) (10). In some PMN experiments a slightly different Boyden type chamber was used (11) so that the lower compartment could be aspirated and the number of cells which had migrated into the lower chamber counted.

Random PMN mobility was assessed using the capillary tube migration assay (12). PMNs were isolated by combined plasmagel sedimentation and Ficoll-Hypaque centrifugation. Capillary

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[1] *Abbreviations used in this paper:* BF, bacterial factor; CIA, chemotactic inhibitory activity; DTT, dithiothreitol; HBSS, Hanks' balanced salt solution; MEM, minimum essential medium; NS, normal human serum; PBS, phosphate-buffered saline; PMN, polymorphonuclear leukocyte.

tubes were incubated in chambers containing minimum essential medium [Eagle] (MEM) supplemented with 5% fetal calf serum. The index of random mobility was the product in millimeters of the two axes of the developing cell flare. The index for control cells ranged from 7.5 to 16 depending on the donor.

Chemotactic factors used in this study consisted of a 10% solution of fresh normal human serum (NS), a 10% solution of culture supernate from a 24-h culture of *Escherichia coli* (strain K 12) grown in MEM and referred to as bacterial factor (BF), a 20% solution of casein-saturated MEM (approximately 2 mg/ml) (7), and purified C5a (13). In each case as specified, test samples were added either to the upper or lower compartment of the chemotaxis chamber. Resulting chemotaxis toward a control chemotactic attractant in the presence of the test sample was compared to that obtained with only the control chemotactic attractant. The effects of the test sample were expressed as a percent of the control. The mean chemotactic index for each of the chemotactic factors used was as follows: NS = 81 ± 31; BF = 76 ± 37; casein = 83 ± 17. These indices are in the optimum range for detection of effects on chemotaxis in this system.

Testing of Sera, M Components, and Colostral IgA. Whole sera from patients with IgG and IgA myeloma, as well as sera from patients with Waldenström's macroglobulinemia, were tested for inhibition in chemotaxis assays before and after heat treatment at 56°C for 30 min. This treatment has previously been shown to enhance CIA in various test sera, possibly as a result of aggregation of inhibitors or inactivation of inhibitor antagonists (7).

IgA M components were prepared from the sera of patients with IgA myeloma containing greater than 1,500 mg/100 ml IgA by a combination of starch block electrophoresis and DEAE ion exchange chromatography (14). In some cases IgA preparations were further fractionated over Sephadex G-200 gel equilibrated with pH 7.4 phosphate-buffered saline (PBS) or by passage through an insolubilized anti-IgG immunoadsorbent column (15). Immunoelectrophoresis of isolated IgA M components showed only a single precipitin band corresponding to IgA with anti-whole human serum. No reactions were noted with several antisera to IgG. Several IgA myelomas which had been previously typed as IgA_1 or IgA_2 were provided by Doctors H. G. Kunkel, H. Grey, R. Wistar, and H. Spiegelberg. Preparations of purified human colostral IgA were obtained from pooled human colostrum (16).

Serum concentrations of IgA were determined using the Oudin tube technique (17). When isolated preparations of IgA were used, the concentrations were determined by optical density at 280 nm or by the Folin reaction (18).

Immunoadsorbent Columns. Insolubilized immunoadsorbent columns were prepared by cyanogen bromide conjugation of either rabbit anti-IgG or anti-IgA to sepharose (15).

Ultracentrifugation. Ultracentrifugation for removal of large immunoglobulin complexes was achieved using a Beckman preparative model L ultracentrifuge and centrifugation (Beckman Instruments, Inc., Fullerton, Calif.) at 100,000 *g* for a period of 1½ h.

5–20% sucrose density gradient centrifugation of whole serum was performed as previously described (2) using an SW-50.1 swinging bucket rotor and centrifuging at 100,000 *g* for 15 h. In some cases 0.2 ml of isolated IgA preparations (8–10 mg/ml) were separated on 7-ml gradients using a SW 41 swinging bucket rotor and 18 h of centrifugation at 100,000 *g*. After centrifugation, 0.3-ml fractions were collected from the bottom, dialyzed against PBS, and tested for CIA.

Pepsin Digestion of IgA. IgA (10 mg/ml) was incubated at 37°C with pepsin (2% by weight) at pH 4.1 (0.1 M acetate) for 18 h (19). The sample was then dialyzed against PBS or passed through a Sephadex G-200 column equilibrated with PBS. Eluted protein peaks were concentrated to original volume. Each preparation was assayed for IgA or IgA fragments by Ouchterlony immunodiffusion analysis and found to be devoid of Fc antigenic determinants.

Reduction and Alkylation of IgA M Components. Isolated IgA M components (8–10 mg/ml) were reduced with dithiothreitol (DTT) (0.01 M) for 1 h at 37°C, alkylated for 15 min in the dark at room temperature with 0.033 M iodoacetamide (20), and analyzed by polyacrylamide gel electrophoresis (21).

Results

Effects of Sera from Patients with IgG Myeloma, IgA Myeloma, or Waldenström's Macroglobulinemia on Normal PMN Chemotaxis. 12 sera from patients with IgG myeloma, 24 patients with IgA myeloma, and 5 patients with

Waldenström's macroglobulinemia were tested before and after a 56°C, 30-min heat treatment for their effects on normal PMN chemotaxis toward 10% fresh normal serum. The test sample consisted of a mixture of 10% normal serum and 10% patient serum. No general suppression of PMN chemotaxis was observed when IgG myeloma or Waldenström's macroglobulinemia sera were tested (Fig. 1). However, when 24 IgA myeloma sera were tested in a similar manner, inhibition was observed with the majority of samples before heat treatment and in all but three samples after 56°C heat treatment. The mean effect of the IgA myeloma sera before heat treatment was a 43% suppression of chemotaxis and 71% inhibition after heat treatment. This suppression occurred with both PMN preparations obtained by plasmagel sedimentation of erythrocytes that contained mononuclear cells and PMN preparations obtained by a combination of plasmagel sedimentation followed by Ficoll-Hypaque centrifugation to remove mononuclear cells. There was no apparent association between serum concentrations of IgA or IgA subclasses or light chain type and the degree of chemotactic inhibition (Table I). These experiments were reproduced in a modified version of the Boyden chemotaxis chamber which allows aspiration of the lower compartment fluid (11), so that PMNs which may fall off the lower side of the membrane could be quantitated. Results indicated that the number of cells found in the lower compartment were greatly reduced when IgA myeloma sera were added to the chemotaxis system. Thus, reduced adherence of PMNs to the lower side of the membrane was not responsible for the observed reduction in the chemotactic index.

Suppression of Casein and BF-Medicated Chemotaxis by IgA Myeloma Sera. 10 IgG myeloma sera, 7 IgA myeloma sera, and 4 Waldenström's macroglobulinemia sera were tested simultaneously with respect to their effect on chemotaxis mediated by NS, casein, and BF. Fig. 2 shows that although no CIA was observed in IgG myeloma samples when NS was used as a chemotactic attractant, some suppression was observed when BF or casein were used as chemotactic attractants. The mean inhibition was 25% for tests using BF and 14% for tests using casein. Although some inhibitory activity was found in IgG myeloma samples, this did not compare to that found in IgA myeloma samples in degree or in frequency. The mean inhibitory activity observed with the seven IgA myeloma sera was 75% when NS was used as a chemotactic attractant, and 78% and 77% when BF and casein were used as chemotactic attractants. One of these IgA myeloma samples failed to suppress NS-mediated chemotaxis. This lack of CIA was consistent with all three chemotactic factors. The suppression of chemotaxis by IgA myeloma sera was nonspecific with respect to chemotactic attractants since inhibition was of the same order of magnitude with each chemotactic factor.

Effects of Isolated IgA M Components and Colostral IgA on PMN Chemotaxis. Because CIA appeared to be most pronounced in IgA myeloma sera, isolated IgA myeloma components were added to the 0.4 ml upper compartment of the chemotaxis chamber and titrated with respect to suppression of PMN chemotaxis. Suppression was observed at concentrations as low as 0.0625 mg of the isolated M component, and inhibition was independent of the chemotactic factor used (Fig. 3). A total of 11 isolated IgA M components including the two shown in Fig. 3 and 1 pooled human colostral IgA preparation were tested both

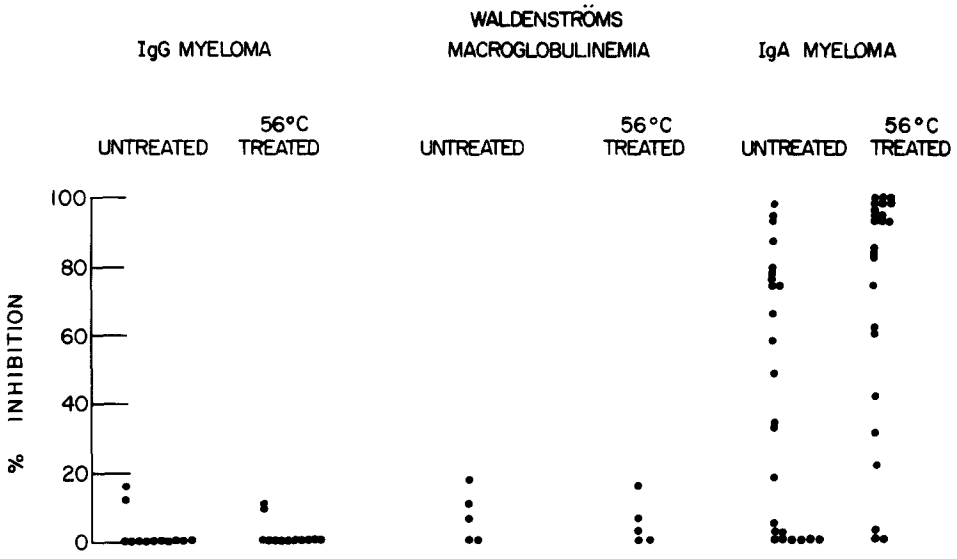

FIG. 1. Effects of IgG and IgA myeloma sera and Waldenstrom's macroglobulinemia sera on PMN chemotaxis toward fresh NS. Results indicate effect before and after 56°C heat treatment of the test sample.

before and after a 30 min, 56°C heat treatment with respect to PMN CIA (Fig. 4). This heat treatment tended to enhance the observed CIA. Both isolated IgA M components and isolated human colostral IgA effectively suppressed PMN chemotaxis, indicating that IgA-mediated suppression of chemotaxis was not associated solely with myeloma IgA. Four of the isolated IgA M components were also tested in a PMN chemotactic system mediated by purified C5a and similar suppression was recorded. Tests using similar concentrations of two preparations of pooled human IgG (human fraction II) and three isolated IgG M components resulted in a mean inhibition of 28 ± 7% before heat treatment and 23 ± 13% after heat treatment. This inhibitory activity was significantly less than that observed with IgA preparations (mean inhibition = 59 ± 36% before heat treatment and 83 ± 28% after heat treatment).

Effects of IgA Myeloma Serum, Isolated IgA M Components, and Colostral IgA on Monocyte Chemotaxis. Table II shows the comparative results of monocyte and PMN chemotaxis experiments where 0.1 ml of heat-treated IgA myeloma serum or 1 mg of isolated IgA M component or colostral IgA were added to the upper compartment of the Boyden type chamber. Very little suppression of monocyte chemotaxis was observed when whole myeloma sera were used, and partial suppression of monocyte chemotaxis was observed when isolated components were used.

Loss of CIA After Absorption of IgA by Specific Immunoadsorbent Columns. 0.5-ml samples containing 6 mg of isolated IgA from two patients with demonstrable CIA were passed through a Sepharose anti-IgA immunoadsorbent column. Eluted fractions were concentrated to original volume dialyzed against HBSS and tested for CIA both before and after a 56°C, 30 min heat treatment. No IgA was present in these fractions as determined by immunodiffusion analysis nor was CIA observed.

TABLE I
Effects of Serum from Patients with IgA Myeloma on Normal PMN Chemotaxis

Test* serum sample	Myeloma subclass	Light chain class	IgA concentration	Inhibition of chemotaxis	
				Before heat treatment	After heat treatment
			mg/100 ml	%	
IgA myeloma					
1	–	–	2,500	34	98
2	–	κ	1,480	66	93
3	–	λ	1,700	78	74
4	–	λ	1,580	0	0
5	–	κ	2,250	18	62
6	–	–	2,220	74	94
7	IgA_1	κ	1,280	87	95
8	–	λ	1,750	79	99
9	IgA_1	λ	2,200	49	99
10	IgA_1	κ	1,720	0	3
11	IgA_1	κ	1,500	33	98
12	–	κ	4,100	0	94
13	IgA_1	λ	1,330	97	98
14	IgA_1	λ	2,320	76	92
15	IgA_1	κ	2,780	94	92
16	IgA_1	κ	2,320	2	82
17	IgA_1	κ	1,480	0	31
18	IgA_2	κ	1,800	58	71
19	IgA_2	λ	1,030	2	42
20	–	λ	1,950	74	83
21	–	κ	2,140	5	85
22	IgA_1	κ	1,950	98	99
23	IgA_1	κ	1,450	0	0
24	IgA_2	κ	1,370	0	22
				Average 43	71

* In each case 10% fresh NS was used as a control chemotactic attractant and the test sample consisted of a mixture of 10% fresh human serum and 10% untreated or heat-treated (56°C 30 min) patient serum.

Site of IgA Chemotactic Suppression. Experiments were conducted to determine if the observed suppression of PMN chemotaxis was due to a direct cellular effect. PMNs (5.6×10^6) were incubated for 15 min at 37°C in 0.5 ml of a preparation of isolated IgA M component (2 mg/ml). Cells were washed twice and tested for their chemotactic responsiveness. Controls consisted of cells treated similarly with PBS and tested in the presence or absence of IgA M component. Preincubation of PMNs with two isolated IgA M components and subsequent washing resulted in a 84 and 100% suppression of chemotaxis, while the direct addition of the M component to the chemotaxis chamber resulted in a 92 and 100% inhibition of chemotaxis, respectively. These data support a cellular mode of chemotactic inhibition. In additional experiments this cell-directed inhibition of PMN chemotaxis was found to be partially reversible after a 3-h subsequent incubation of PMNs in MEM supplemented with 5% heat-inactivated fetal calf serum as shown in Fig. 5.

Effect of IgA M Component on PMN Random Mobility. PMN random

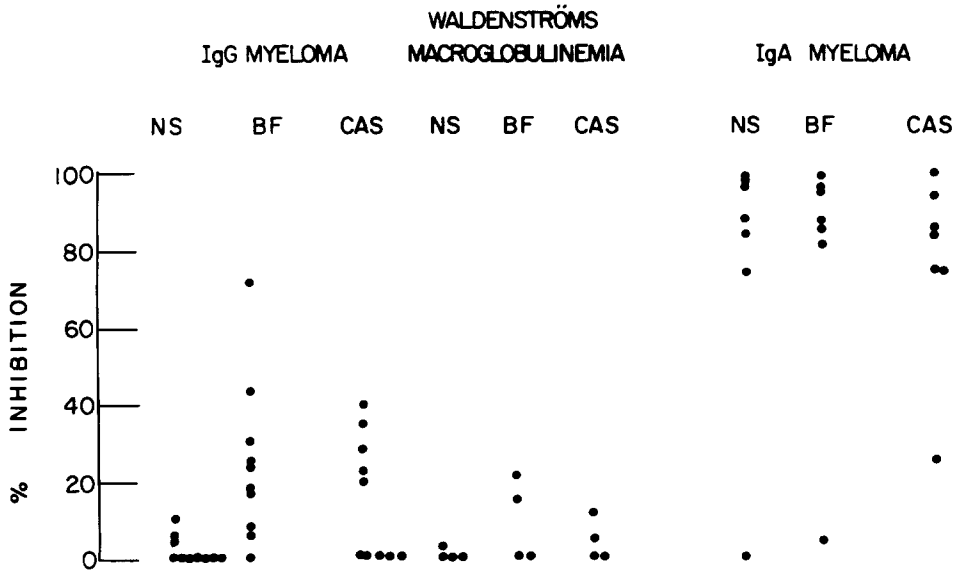

FIG. 2. Effect of IgG and IgA myeloma sera and Waldenström's macroglobulinemia sera on PMN chemotaxis toward human serum (NS), bacterial-derived chemotactic factor (BF), and casein (Cas). Test samples were heat treated (56°C for 30 min) and used at 10% concentrations in the lower compartment of the chemotaxis chamber with each chemotactic factor.

mobility was assayed by the capillary tube migration technique (12). In each case capillaries containing PMNs were fixed into chambers and incubated in control medium or medium containing 1 mg/ml of isolated IgA M component. The results in Table III show that in three cases (IgA's no. 2, 3, and 6) partial suppression of random mobility was observed although the degree of inhibition was much less than the suppression of PMN chemotaxis, while in two cases IgA samples with greater than 80% CIA failed to suppress random mobility.

Physical Studies

ULTRACENTRIFUGATION AND SUCROSE DENSITY GRADIENT CENTRIFUGATION. Since heat treatment resulted in enhanced CIA, it was postulated that aggregates of IgA may interact with the PMN-IgA receptor (1) and result in chemotactic suppression much like that mediated by immune complexes (22). Two IgA myeloma serum samples were centrifuged at 100,000 *g* for $1^1/_2$ h to remove any large aggregates of IgA that might exist in these samples. Tests of these samples following this procedure indicated that CIA remained in the supernate, while the small 0.2 ml pellet of material at the bottom of the tube showed no CIA.

IgA myeloma serum samples and M components were separated by 5–20% sucrose density gradient centrifugation, and gradient fractions were tested for CIA. As shown in Fig. 6 A, B, and C the majority of inhibitory activity in three different IgA myeloma sera was found to sediment within the 10S to 14S region as compared to the 7S human IgG marker. However, some inhibitory activity was also found in the 7S to 8S region in certain samples.

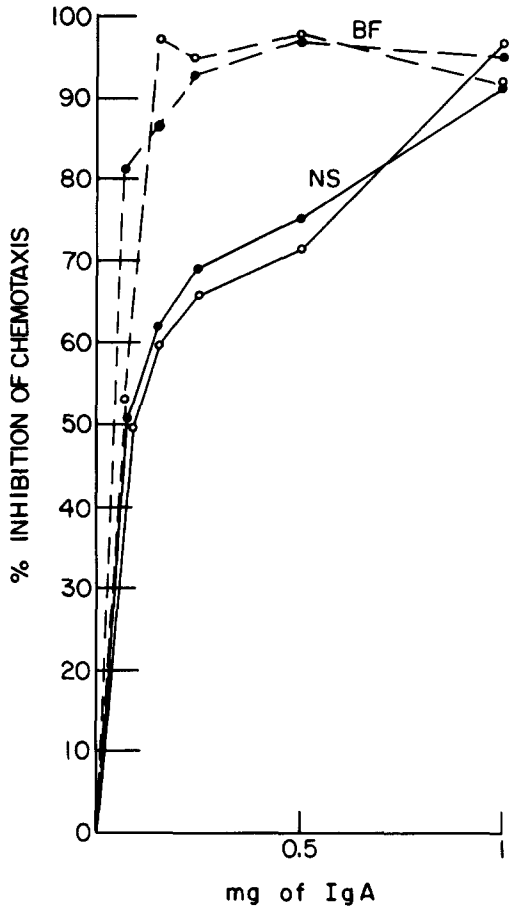

FIG. 3. Titration of isolated IgA M components with respect to inhibitory effects on BF-mediated PMN chemotaxis (---) and NS-mediated PMN chemotaxis (—). The control chemotactic indices for NS-mediated chemotaxis were 68 and 73 and for BF-mediated chemotaxis were 102 and 40.

In addition, isolated IgA M components from three different patients were centrifuged on 5–20% sucrose density gradients (Fig. 6 D, E, and F). 0.1 ml of each gradient fraction was added to the upper compartment of duplicate Boyden chambers and tested for its effect on PMN chemotaxis toward fresh NS. Multiple peaks of CIA were found in fractions sedimenting between 7S and 13S, Fig. 6 B, in regions where polymeric forms of IgA would be expected to sediment.

EFFECTS OF PEPSIN TREATMENT AND DTT REDUCTION AND ALKYLATION ON IgA INHIBITION OF CHEMOTAXIS. Pepsin-treated IgA M components (0.5 mg) were tested for CIA and compared to control untreated IgA M components subjected to similar pH and temperature conditions. Results show that although isolated IgA M components suppressed PMN chemotaxis, a substantial loss of CIA was observed when these M components were pepsin digested (Table IV, A). Similarly, when $F(ab)_2$ fragments were isolated by G-200 fractionation, no CIA was detected.

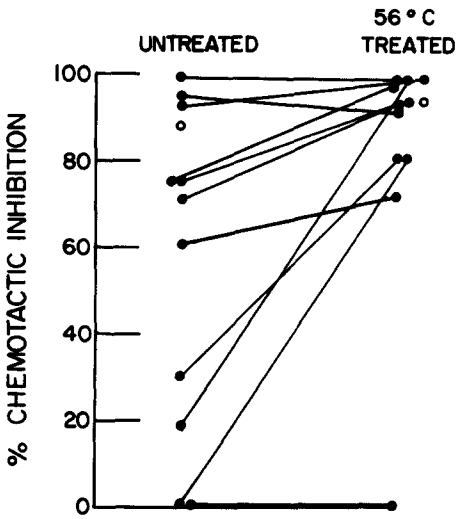

FIG. 4. Results of chemotaxis in the presence of isolated IgA M components (closed circles) and pooled human colostral IgA (open circles). In each case IgA was added to the cell compartment and chemotaxis was toward fresh NS. Lines connect the points on the graph representing the same sample before and after heat treatment.

TABLE II

Effect of Myeloma and Colostral IgA on Monocyte and PMN Chemotaxis

Sample	Inhibition* of PMN chemotaxis	Inhibition‡ of monocyte chemotaxis
	%	%
Exp. A. Whole IgA myeloma sera		
1	99	19
2	71	10
3	92	0
Exp. B. Isolated IgA M components (1 mg)		
1	81	51
2	98	18
3	91	29
4	93	52
Colostral IgA (1 mg)	93	44
	Mean = 90	Mean = 28

* Inhibition using 10% fresh NS as a chemotactic attractant.

‡ Inhibition using a mixture of casein and fresh NS as a chemotactic attractant. Control levels for Experiment A and B were 75 and 36 monocytes per oil immersion field.

Four isolated IgA M components capable of suppressing PMN chemotaxis before heat treatment were reduced with DTT, alkylated, and subsequently tested for PMN CIA. This treatment resulted in both a conversion of the majority of polymeric IgA to its monomeric form as evidenced by polyacrylamide gel electrophoresis and a simultaneous reduction in CIA (Table IV, B).

EFFECT OF POLYMERIC AND MONOMERIC IgA ON PMN CHEMOTAXIS. An isolated IgA preparation containing both polymeric and monomeric forms of IgA was

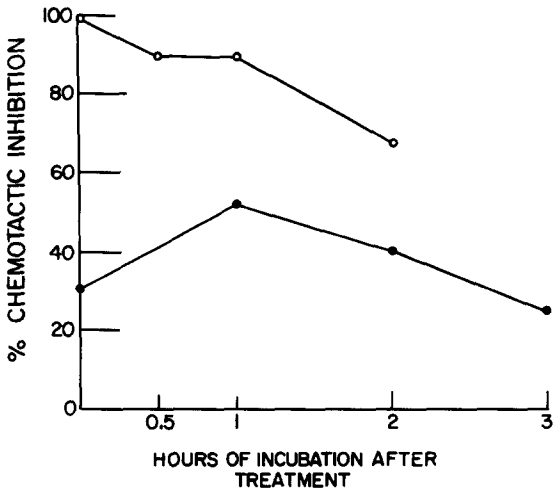

FIG. 5. Reversible inhibition of IgA-mediated chemotactic suppression. Results with two different IgA M components using 10% fresh normal serum as a chemotactic factor are shown. The initial control chemotactic indices for each experiment were 86 and 140. These control values varied by less than 20% throughout the 3 h of incubation.

TABLE III

Effect of IgA M Components on PMN Random Mobility as Compared to Chemotaxis

Sample	Random* mobility index	Inhibition of chemotaxis
		%
Experiment A		
Control	9.0 (0%)	–
IgA no. 1	9.0 (0%)	0
IgA no. 2	6.25 (31%)	81
Experiment B		
Control	7.5 (0%)	–
IgA no. 3	5 (33%)	98
IgA no. 4	7.5 (0%)	96
IgA no. 5	7.5 (0%)	82
Experiment C		
Control	16 (0%)	–
IgA no. 6	9 (44%)	93

* Number in parentheses indicates the percent suppression of random mobility.

fractionated on Sephadex G-200 (Fig. 7). Fraction 38 containing 0.2 mg/ml of protein from the exclusion volume and fraction 46 from the second peak were dialyzed against HBSS. 1 ml of fraction 38 was reduced with DTT and alkylated with iodoacetamide. Each of the three samples were subsequently assayed by polyacrylamide gel electrophoresis. Multiple bands were observed in fraction 38 which represent various polymeric forms of IgA with one band representing a small amount of monomeric IgA (Fig. 7). After reduction and alkylation of this

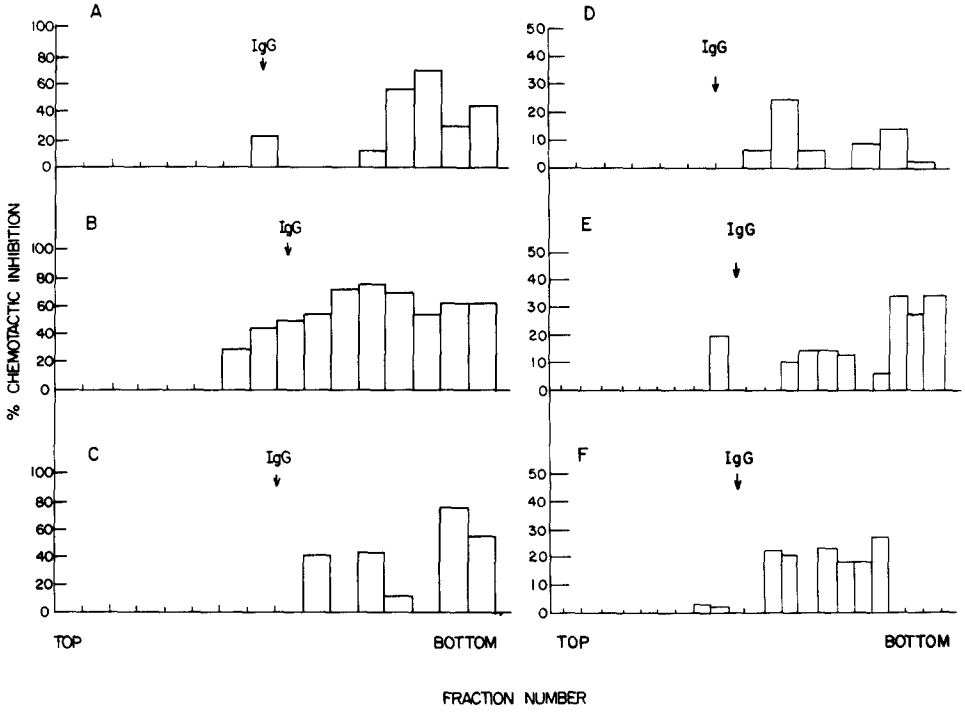

FIG. 6. Separation of IgA myeloma sera and isolated IgA M components by sucrose density gradient centrifugation. Parts A, B, and C are results on whole IgA myeloma serum; parts D, E, and F are results with isolated IgA M components. Chemotaxis was toward 10% fresh normal serum. In each case the position of the 7S IgG marker is indicated.

sample, a single band corresponding to the monomeric form of IgA was observed. Acrylamide gel analysis of fraction 46 indicated that the majority of protein in this fraction was monomeric IgA with a small amount of polymeric IgA carried over from the exclusion volume peak. These same samples were tested with respect to their effect on PMN chemotaxis. PMNs (7×10^6) were suspended in either 1 ml of HBSS, fraction 38, fraction 38 reduced and alkylated, or fraction 46. Cell preparations were then tested for chemotactic responsiveness toward fresh normal serum. Fig. 7 demonstrates that fraction 38 containing polymeric IgA effectively suppressed PMN chemotaxis. This suppressive activity was lost after reduction and alkylation which resulted in a conversion to the monomeric form of IgA. Likewise, fraction 46 containing primarily monomeric IgA and only small proportions of polymeric IgA failed to inhibit PMN chemotaxis.

Discussion

From this study, it is evident that sera from patients with IgA myeloma contain potent suppressors of PMN chemotaxis. CIA was most prominent in the IgA class of myeloma in systems using fresh NS since IgG myeloma and Waldenström's macroglobulinemia sera generally failed to inhibit PMN chemotaxis either before or after a 56°C heat treatment. Inhibition of PMN chemotaxis by IgA myeloma samples was not specific to a single chemotactic factor, and

TABLE IV
Effect of Pepsin Digestion and Reduction-Alkylation on IgA M Component Suppression of PMN Chemotaxis

Sample*	Inhibition of Chemotaxis
	%
A. Pepsin digestion	
IgA no. 1	66
IgA no. 1 – pepsin digested	30
IgA no. 2	50
IgA no. 2 pepsin digested	28
B. Reduction – alkylation	
IgA no. 1	57
IgA no. 1 reduced and alkylated	19
IgA no. 2	47
IgA no. 2 reduced and alkylated	4
IgA no. 3	97
IgA no. 3 reduced and alkylated	0
IgA no. 4	36
IgA no. 4 reduced and alkylated	22

* 10% normal serum was used as a chemotactic attractant, and 0.5 mg of the IgA preparation was added to the upper compartment.

equivalent inhibition was observed with NS, C5a, BF, or casein-mediated chemotaxis. Some CIA was noted with whole heat-treated IgG myeloma sera when BF or casein were used as chemotactic attractants, but this suppressive activity did not compare to that observed in the IgA myeloma samples either in degree or frequency. This inhibitory effect may be the result of IgG aggregates or the presence of the heat stable cell-directed chemotactic inhibitor recently described in patients with sarcoma and carcinoma (23). Failure of IgG myeloma samples to suppress the normal serum-mediated chemotactic system was probably due to the intrinsic capacity for IgG to activate complement, thereby generating complement-derived chemotactic factors.

Analysis of IgA myeloma sera failed to show any parallel between CIA and the subclass of IgA, light chain type, or the absolute concentration of IgA, although when isolated IgA M components were tested, CIA was found to be directly associated with these components. Sucrose density gradient centrifugation indicated that the majority of CIA sedimented in the 10 to 14S range. Maximal inhibitory activity in these fractions implied a possible involvement of polymeric forms of IgA and was consistent with both the enhanced CIA observed after 56°C heating of IgA myeloma samples and chemotactic inhibition by colostral IgA. Similarly, these findings appeared to explain the discrepancy between absolute IgA M component concentration and levels of CIA.

Further support for the involvement of polymeric IgA was derived from experiments that demonstrated substantial loss in IgA-mediated chemotactic inhibition after DTT sulfhydryl reduction and alkylation which results in con-

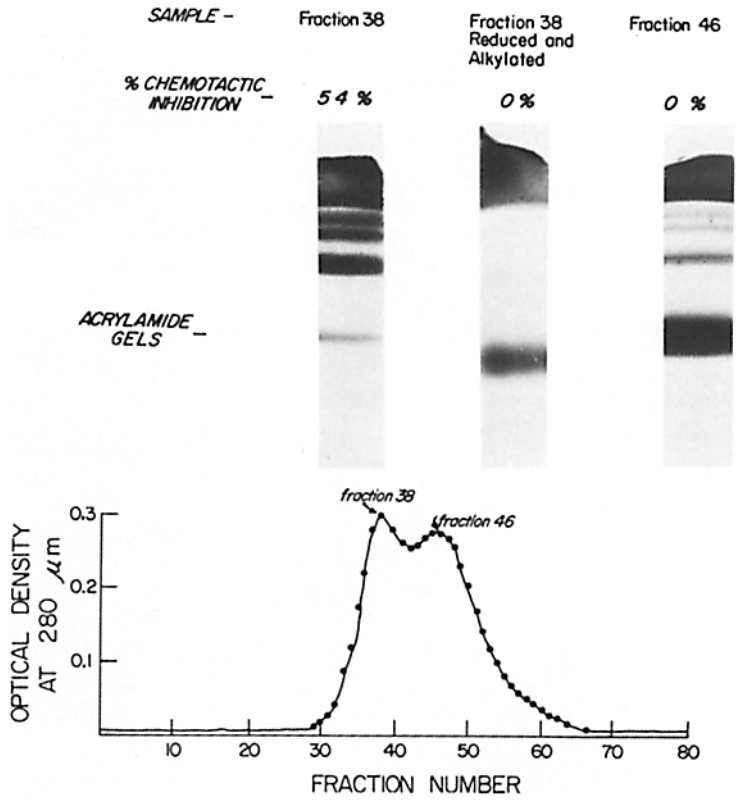

FIG. 7. Association of inhibitory activity with exclusion volume fractions after separation of IgA M component by Sephadex G-200 gel filtration. Results of chemotaxis with peak fractions before and after reduction alkylation and polyacrylamide gel analysis of these fractions are shown.

version of polymeric forms of IgA to their monomeric subunits (20). Additional evidence supporting polymeric forms of IgA as chemotactic inhibitors was obtained by Sephadex G-200 gel filtration of isolated M components. Exclusion volume IgA suppressed PMN chemotaxis at low concentrations and lost this suppressive activity when samples were converted to their monomeric counterparts by reduction and alkylation. Taken together, these data indicate that high molecular weight IgA is responsible for the majority of CIA. It is pertinent to note that although the majority of CIA was present in the 10 to 14S regions, some 7–8S preparations also showed CIA (Fig. 6).

The mode of IgA suppression of PMN chemotaxis appeared to be cell directed since incubation of PMNs with IgA M components and subsequent washing resulted in a suppressed chemotactic response. This cell-directed inhibition was demonstrated to be at least partially reversible when PMNs were cultured in medium supplemented with fetal calf serum. Although slight effects on random PMN mobility were recorded with some IgA M components, such effects did not parallel the degree of chemotactic inhibition observed.

IgA inhibitory activity was dependent on the Fc portion of the molecule since a loss in CIA was observed after pepsin digestion. In addition, data indicate that

polymeric IgA was a more effective inhibitor than monomeric IgA, and that PMNs were effected to a greater extent than monocytes. These data parallel the IgA binding studies reported by others (1, 24). Furthermore, three of the IgA preparations studied here were generously provided by Dr. Hans Spiegelberg and were previously found to bind to human PMNs using radioactive binding techniques (1, 24). These data imply that chemotactic inhibition and IgA binding may be mediated by similar membrane receptors. This type of chemotactic suppression resembles that described with IgG antibody-antigen complexes (22), although in the current study it was apparent that IgA was a more effective suppressor than IgG. Differences between IgG and IgA suppression may be the result of interaction with unique and separate PMN immunoglobulin receptors (1) or alternatively, due to the inherent ability of IgA to polymerize and form a more reactive complex.

It is possible that degranulation resulting from PMN interaction with IgA (24) may be related to the observed chemotactic inhibition. Although degranulation is a potential mechanism of chemotactic inhibition, our current evidence indicating partial reversibility of the IgA inhibitory effect after prolonged incubation is not entirely consistent with this concept.

Data presented here support three conceivable mechanisms of IgA-mediated chemotactic inhibition. First, (part A, Fig. 8) chemotactic inhibition may be the result of interaction with the PMN IgA receptor which could directly inhibit PMN chemotactic receptors or suppress cellular mechanisms essential for chemotaxis. A second possible mode of inhibition (part B of Fig. 8) assumes that since IgA may be bound to many other serum components (25–27), CIA may be the result of these associated components. Such inhibition could result from steric hindrance, direct inactivation of chemotactic factor before interaction with the cell membrane, or suppression of cellular functions essential for chemotaxis. Finally, (Fig. 8 c) interaction with the PMN may be mediated by a component associated with IgA which could then result in chemotactic inhibition by any of the previously described mechanisms.

It is apparent from these data that IgA immunoglobulin may interact with the PMN and result in a suppression of the chemotactic response. Furthermore, since previous in vitro data show that IgA does not activate complement via the classic complement pathway, and may effectively block serum bactericidal activity (28), it is possible that in vivo this immunoglobulin may modify various aspects of the inflammatory response.

Summary

Sera from patients with IgA myeloma suppressed polymorphonuclear leukocyte (PMN) chemotaxis, while generally little or no suppression was observed with sera from patients with IgG myeloma or Waldenström's macroglobulinemia. Chemotactic inhibitory activity was not limited to a single chemotactic factor and was equivalent when C5a, bacterial factor, casein, or normal serum were used as chemotactic attractants. No association was noted between the degree of inhibitory activity and the IgA subclass or light chain type.

Chemotactic inhibitory activity was found to be directly associated with isolated IgA M components, and similar chemotactic suppression was noted with

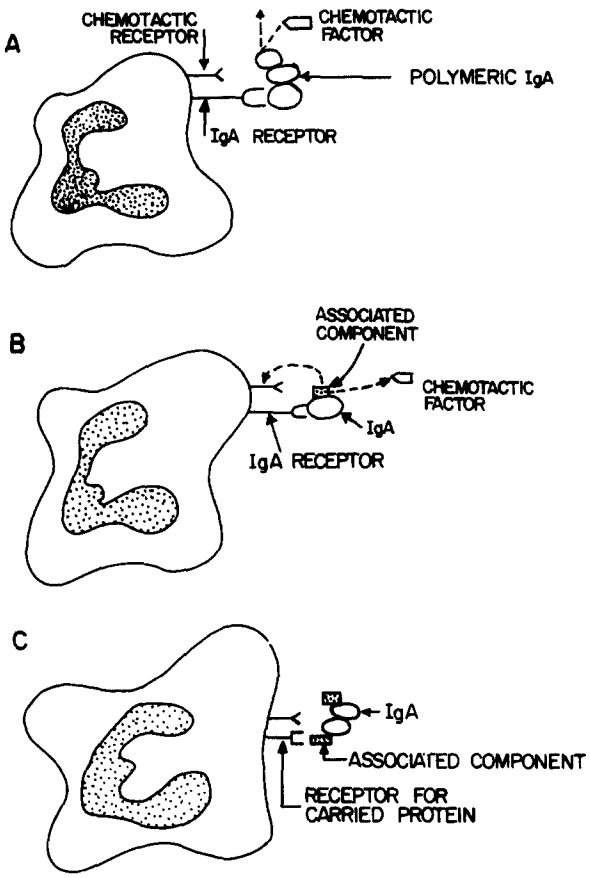

FIG. 8. Three possible mechanisms of chemotactic inhibition by IgA.

purified preparations of normal human colostral IgA. By comparison, IgA preparations were most effective in suppressing PMN chemotaxis and had a much lesser effect on monocyte chemotaxis. The mode of IgA chemotactic inhibition was cellular and at least partially reversible after a 37°C incubation in the absence of IgA. Some inhibition of PMN random mobility was noted with certain IgA preparations, although such effects did not parallel the degree of chemotactic inhibition.

Fractionation of IgA myeloma sera and IgA M components by sucrose density gradient centrifugation showed multiple peaks of inhibitory activity in 10 to 13S fractions. The majority of IgA inhibitory activity was lost after pepsin digestion or sulfhydryl reduction and alkylation of isolated M components. When isolated IgA M components were fractionated on Sephadex G-200, inhibitory activity was associated with the exclusion volume and was abolished by reduction and alkylation procedures which resulted in a conversion of polymeric IgA to monomeric IgA.

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