INFECTION AND IMMUNITY, Feb. 1989, p. 452–457
0019-9567/89/020452-06$02.00/0

Vol. 57, No. 2

Chemotactic Properties of Rat Immunoglobulins and Immune Complexes

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Received 25 May 1988/Accepted 27 October 1988

The effect of rat immunoglobulins and immune complexes on the locomotor function of rat polymorphonuclear leukocytes (PMN) was investigated in vitro. Rat immunoglobulin G1 (IgG1), IgG2a, IgG2b, and IgA monoclonal antibodies specific for the dinitrophenyl hapten were used. Both monomeric and polymeric IgA showed chemotactic activity in a dose-dependent manner. IgG1 and IgG2b also induced a dose-dependent locomotor response of PMN, but the nature of the induced migration was chemokinetic (enhancing random migration). IgG2a was chemotactic and induced maximal migration at a relatively low concentration. IgG1- and IgG2b-immune complexes induced stronger migration than antibody alone; however, IgA- and IgG2a-immune complexes did not. IgA was shown to modify the chemotactic movement of PMN induced by *N*-formylmethionyl-leucyl-phenylalanine (FMLP). In the presence of both IgA and FMLP in the lower chamber, the migration towards suboptimal concentrations of FMLP was enhanced. By contrast, IgA in the upper chamber decreased migration towards the optimal or higher concentrations of FMLP. These findings suggest that IgA may work synergistically with luminal chemoattractants to mobilize PMN to the locus of infection on the mucosal surface. In addition, the intense activity of IgG2a alone and IgG1- or IgG2b-immune complexes in inducing PMN migration may play an important role in inflammatory processes. The data indicate that immunoglobulins have a direct effect on PMN mobility.

Immunoglobulins can interact with polymorphonuclear leukocytes (PMN) (16) to modify various effector functions, including phagocytosis (18), release of lysozomal enzymes (11), respiratory burst (9), and antibody-dependent cell-mediated cytolysis (17). Immunoglobulins or immune complexes are considered to induce PMN migration chiefly through the activation of complement, with subsequent liberation of the chemotactic peptide C5a (15, 23, 26). Although the complement mechanism is clear, the effect of immunoglobulin molecules on neutrophil migration is controversial. For example, in three studies (14, 24, 25), human polymeric or aggregated immunoglobulin A (IgA) had a suppressive effect on chemotactic movement of PMN towards various chemoattractants, including *N*-formylmethionyl-leucyl-phenylalanine (FMLP). However, Sibille et al. (22) observed that human IgA induced chemokinetic PMN movement, and when IgA was mixed with suboptimal concentrations of FMLP, the chemotactic activity of this peptide was significantly enhanced. Polyclonal IgG has been found to have no effect on PMN movement (28) or to stimulate chemotaxis (22), and heat-aggregated IgG was found to stimulate migration (28) and facilitate PMN sticking (13, 29) and thereby reduce PMN migration when bound to chemotaxis membranes. Thus, the effect of immunoglobulin molecules (as native proteins, aggregates, or immune complexes) on PMN migration is uncertain.

Rits et al. (20, 21) have developed several clones of rat hybridomas which secrete IgG or IgA monoclonal antibodies against the dinitrophenyl (DNP) hapten. These reagents have enabled us to investigate the influence of purified rat immunoglobulins and immune complexes on PMN migration. By using these antibodies, the effect of the three major rat subclasses of IgG (IgG1, IgG2a, and IgG2b) and monomeric and polymeric IgA on the locomotor function of rat PMN has been investigated. The results show that both IgG and IgA induce the migration of PMN. Moreover, the nature of the induced migration (chemotactic or chemokinetic) differs between classes and even between IgG subclasses. Finally, soluble immune complexes containing IgG1 and IgG2b induce stronger migration than antibody alone. Because of the purity of these reagents and the care taken to ensure the molecular species of each monoclonal protein tested, these results offer insights into the relationship between immunoglobulin molecules and PMN migration not possible from prior studies.

MATERIALS AND METHODS

Separation of rat PMN from peripheral blood. Peripheral blood was obtained from Wistar male rats, 12 to 16 weeks old (about 250 g), and PMN were purified with a one-step method on a high-density (specific gravity, 1.095) Ficoll-Hypaque gradient, as described by Ferrante and Thong (7). Ficoll-400 was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Hypaque (85%) was generously provided by Winthrop, Inc., London. Briefly, heparinized blood (5 ml) obtained from the tail vein was gently layered onto the Ficoll-Hypaque mixture (3 ml) and centrifuged at 200 × *g* for 50 min at room temperature. After centrifugation, the band of PMN, separated below the mononuclear cells and above the erythrocytes, was gently aspirated, and cells were washed with precooled Hanks balanced salt solution without calcium and magnesium. Residual erythrocytes were lysed in a buffer containing 0.15 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM disodium EDTA (pH 7.4) at 4°C for 3 min. PMN were suspended in 0.5% human serum albumin (HSA) (Institut Mérieux, Lyon, France) in Hanks balanced salt solution with calcium and magnesium (HBSS/HSA). The PMN fraction contained a high proportion of PMN (90.2% neutrophils and 2.3% eosinophils). The mean total number of PMN recovered was 2.4 × 10^6 cells from 5 ml of rat blood. The

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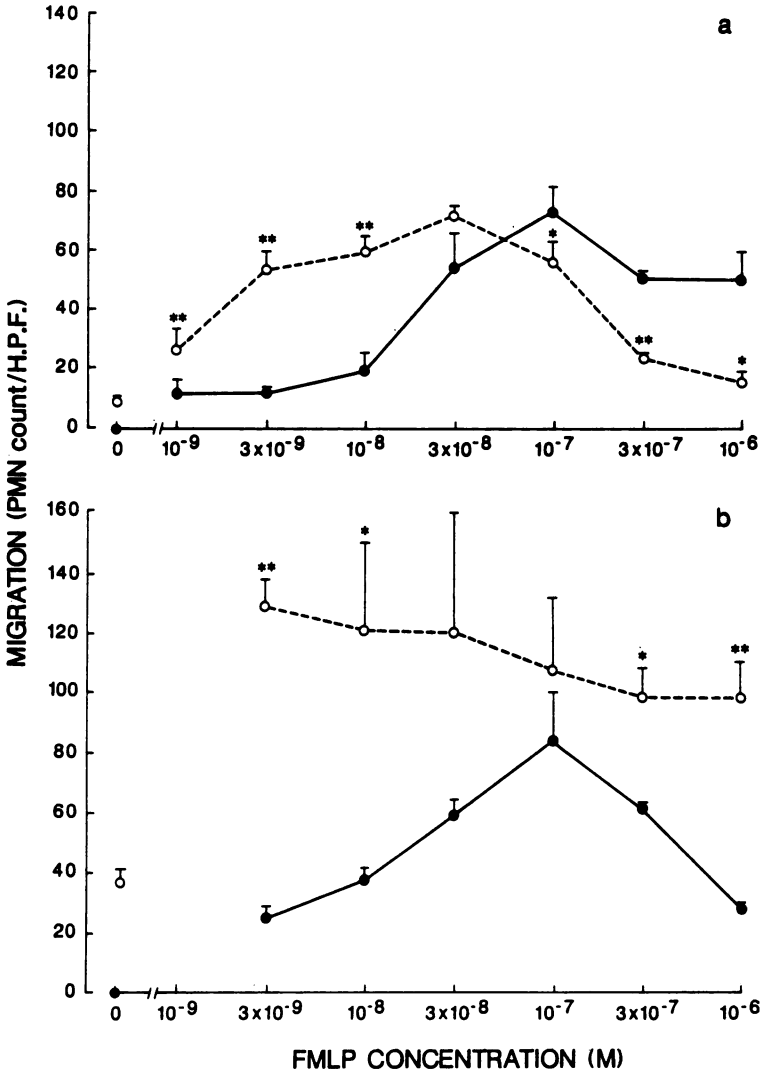

FIG. 1. Effect of IgA on FMLP-induced chemotaxis of PMN. (a) Migration towards FMLP (●) and its change after addition of polymeric IgA (0.3 mg/ml) to the upper chamber (○). (b) Migration towards FMLP (●) and its change after addition of polymeric IgA (0.3 mg/ml) to the lower chamber (○). Degrees of migration (PMN count/H.P.F. after the value for the negative control in each experiment was subtracted) are plotted against the concentration of FMLP. Each point represents the mean ± SD (n = 3). Average values for the negative controls were 2.3 ± 0.5 (a) and 2.7 ± 0.5 (b). **, $P < 0.01$; *, $P < 0.05$ versus migration without IgA.

viability of the purified PMN was 98% as determined by trypan blue dye exclusion.

Sources of rat immunoglobulins. Hybridomas which secreted IgG1 (LO-DNP-2), IgG2a (LO-DNP-16), IgG2b (LO-DNP-65), or IgA antibodies (LO-DNP-45, -58, and -64), all against the DNP hapten, were raised in inbred LOU/c (Louvain) rats. Detailed procedures for establishment and maintenance of these hybridomas were described elsewhere (4, 20). These hybridomas were grown in the rat peritoneal cavity, and monoclonal antibodies were purified from the collected ascitic fluid. Rat polyclonal secretory IgA (sIgA) was purified from pooled bile as described previously (1).

Purification of hybridoma IgG and IgA. Rat monoclonal anti-DNP antibodies were purified by methods reported elsewhere (20, 21), with some modifications. Briefly, ascitic fluid collected from each hybridoma-bearing rat was treated with 50% saturated ammonium sulfate. The centrifuged precipitate was dissolved in and dialyzed against 0.02 M Tris hydrochloride buffer (pH 8.0) containing 0.34 M NaCl and 0.015 M NaN_3. This globulin fraction was filtered on a column of Ultrogel AcA 22 (Industrie Biologique Française, Villeneuve la Garenne, France) for the separation of polymeric IgA from monomeric IgA and IgG. The monoclonal polymeric IgA antibodies (LO-DNP-45 and -64) precipitated in a buffer with low ionic strength, and thus were isolated by dialysis against 0.02 M Tris hydrochloride buffer (pH 8.0). The resulting precipitate was dissolved in 0.015 M phosphate-buffered saline (PBS), pH 7.2. The IgG or monomeric IgA fraction was further purified by affinity chromatography on DNP-lysine conjugated with CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The bound antibodies were eluted with 0.1 M dinitrophenol (pH 7.4) and passed through a Dowex AG1-X8 (BioRad Laboratories, Richmond, Calif.) column in PBS to remove the hapten. Polymeric IgA samples were derived from two clones (LO-DNP-45 and -64), and monomeric IgA samples were derived

TABLE 1. Checkerboard analysis of PMN migration induced by polymeric IgA[a]

IgA concn in upper chamber (mg/ml)	Mean PMN count/H.P.F. ± SD (range) at indicated IgA concn (mg/ml) in lower chamber			
	0.0	0.1	0.3	1.0
0.0		12.4 ± 4.1 (7.4–19.3)	21.0 ± 5.5 (11.4–27.3)	31.9 ± 10.0 (19.9–42.2)
0.1	1.8 ± 1.3 (0.3–2.8)	5.6 ± 2.0 (3.4–7.2)	7.0 ± 2.5 (4.1–8.6)	12.1 ± 1.5 (10.7–13.6)
0.3	1.9 ± 1.2 (0.6–2.6)	3.9 ± 1.9 (2.7–9.7)	7.0 ± 2.3[b] (5.1–9.5)	10.1 ± 3.2 (7.2–13.5)
1.0	3.0 ± 2.4 (0.3–4.7)	5.1 ± 0.4 (4.7–5.4)	5.2 ± 1.0 (4.6–6.4)	7.5 ± 3.1[b] (5.0–10.9)

[a] The value for the negative control for each experiment (n = 3) was subtracted for all values shown. The average value for the negative control was 1.7 ± 1.8 (0.3 to 3.7).
[b] $P < 0.05$ versus migration induced with polymeric IgA present only in the lower chamber.

from two clones (LO-DNP-58 and -64). Purity of the samples was checked by agarose gel electrophoresis and immunoelectrophoresis. Concentrations of purified immunoglobulins were measured by light absorption at 280 nm ($E^{1\ cm,1\%}$ = 13.4 for IgA and 13.8 for IgG). The molecular size of all IgA preparations was determined by sucrose density gradient ultracentrifugation (21). Both polymeric IgA preparations (LO-DNP-45 and -64) consisted of similar proportions of dimers, trimers, and tetramers. The predominant molecular form was trimeric (57.2% for LO-DNP-45 and 53.2% for LO-DNP-64), and the mean calculated molecular weight was 465,000 for both polymeric IgA samples.

Immune complexes. DNP_8-bovine serum albumin (BSA) was prepared as reported previously (21). Monoclonal polymeric and monomeric IgA and IgG antibodies were mixed with DNP_8-BSA and incubated at 37°C for 15 min. Final concentrations of antibodies and antigen were 0.1 and 0.13 mg/ml, respectively. At this ratio, each monoclonal antibody forms soluble complexes with DNP_8-BSA in antigen excess (21). The mixtures were checked for their ability to induce migration of PMN as described below.

Chemotaxis. Locomotor responses of PMN towards immunoglobulin samples or FMLP (Sigma Chemical Co., St. Louis, Mo.) were tested with a 48-well microchemotactic chamber (Neuro Probe, Bethesda, Md.) (5). Briefly, 25-μl samples containing the substance of interest at various concentrations in HBSS/HSA were put in wells of the lower part of the chamber. A polycarbonate filter sheet (pore size, 3 μm; thickness, 10 μm; Nuclepore, Pleasanton, Calif.) was carefully placed on the 48 wells. After the silicon gasket and the upper part were fixed, 50 μl of PMN suspension (10^6 PMN/ml in HBSS/HSA) were placed in corresponding wells of the upper chamber. The chamber was incubated at 37°C for 20 min. After the incubation, the filter was removed and the cells on the upper surface of the filter were scraped off. Thereafter, the filter was fixed in methanol and stained with May-Grünwald-Giemsa solution. PMN reaching the lower surface of the filter were counted in three high-power (500×) microscopic fields (H.P.F.) of each well. The results represent means of triplicate wells. In each experiment, migration towards FMLP (at the optimal concentration) and HBSS/HSA were also checked as positive and negative controls, respectively. The degree of migration of each sample is expressed as number of PMN per H.P.F. after subtracting the mean PMN count in the negative control. It was found that with IgA and IgG2a as chemoattractants, very few PMN migrated through the filter into the lower chamber.

To differentiate chemokinesis from chemotaxis, a checkerboard analysis (30) was performed for polymeric IgA. For monomeric IgA and IgG subclasses and for immune complexes, a single concentration (listed in the tables) was also added to PMN in the upper chamber.

Statistical analysis. Data are presented as the mean ± 1 standard deviation (SD). Statistical comparisons were performed by the paired Student's t test.

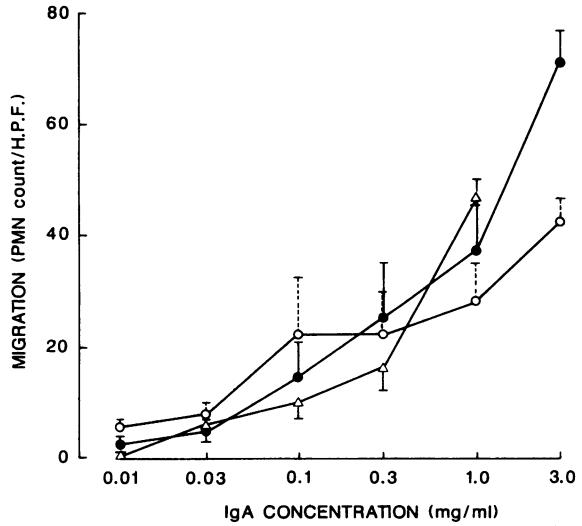

FIG. 2. PMN migration induced by various forms of IgA. Degrees of migration are plotted against concentration of monomeric IgA (○), polymeric IgA (●), and sIgA (△). Each point represents the mean ± SD (for monomeric and polymeric IgA, n = 9; for sIgA, n = 3).

RESULTS

Chemotaxis induced by FMLP. Preliminary experiments showed that HSA could be used as a substitute for rat serum albumin for cell suspension and sample dilution. The chemotactic activity of various concentrations of FMLP (from 10^{-9} M to 10^{-5} M) for PMN was tested. As shown in Fig. 1, FMLP induced maximal chemotaxis at a concentration of 10^{-7} M (79.4 ± 17.4 PMN/H.P.F., n = 31). The mean PMN count of the negative controls was 2.0 ± 1.4 (n = 33).

Influence of immunoglobulins on locomotion of PMN. All preparations of monoclonal polymeric IgA from both LO-DNP-45 and -64 clones induced migration of PMN in a similar dose-dependent manner (Fig. 2). At each concentration, similar degrees of migration were observed with each of the two clones (not shown). When polymeric IgA (0.1 to 1.0 mg/ml) was also present in the upper chamber in a checkerboard analysis, the degree of migration was reduced (Table 1). These data clearly demonstrate that the activity of polymeric IgA on the locomotion of PMN was mainly of the chemotactic type.

Both monomeric IgA preparations (LO-DNP-58 and -64) showed a dose-response curve similar to that of polymeric

TABLE 2. PMN migration induced by monomeric IgA and IgG[a]

Immunoglobulin in upper chamber[b]	Concn (mg/ml)	Mean PMN count/H.P.F. ± SD (range) at indicated IgA or IgG concn (mg/ml) in lower chamber			
		0.0	0.1	0.3	1.0
IgA	0.0		22.1 ± 10.5 (8.7–38.1)	21.9 ± 8.3 (9.4–44.1)	27.9 ± 6.9 (16.7–38.2)
	0.3	0.3 ± 0.7 (0.0–2.8)	2.9 ± 3.0 (0.0–8.7)	3.4 ± 3.2[c] (0.3–10.7)	12.9 ± 3.3 (7.4–18.2)
IgG1	0.0		9.8 ± 0.5 (9.4–10.4)	23.7 ± 2.4 (21.0–25.7)	67.8 ± 9.1 (59.7–77.6)
	0.3	4.7 ± 2.7 (1.7–7.0)	14.2 ± 2.0 (12.0–16.0)	24.4 ± 5.8 (17.7–28.4)	87.5 ± 12.5 (74.4–99.4)
IgG2a	0.0		34.8 ± 15.7 (20.4–54.5)	66.1 ± 11.1 (55.0–84.2)	48.1 ± 6.3 (59.7–77.6)
	0.3	0.0 ± 0.0 (0.0–0.0)	1.1 ± 1.7 (0.0–3.4)	5.3 ± 1.4[c] (3.5–6.9)	9.3 ± 4.9 (1.5–14.7)
IgG2b	0.0		7.1 ± 2.1 (5.3–11.0)	18.7 ± 3.4 (15.0–22.7)	59.5 ± 22.6 (36.0–84.7)
	0.3	3.7 ± 3.3 (0.7–6.7)	11.3 ± 2.4 (8.7–14.3)	20.4 ± 3.6 (17.0–26.0)	69.7 ± 18.2 (46.0–96.3)

[a] See Table 1, footenote *a*. For monomeric IgA, $n = 15$; for IgG1, $n = 3$; for IgG2a and IgG2b, $n = 6$.
[b] Monomeric IgA or the same subclass of IgG was also added to the cell suspension in the lower chamber.
[c] $P < 0.01$ versus migration induced by IgA or IgG2a at 0.3 mg/ml present only in the lower chamber.

IgA; however, the dose dependency was less obvious, and at higher concentrations (1.0 to 3.0 mg/ml), the migration induced by monomeric IgA was weaker than that induced by polymeric IgA (Fig. 2). Monomeric IgA also showed a chemotactic type of activity for PMN (Table 2). Polyclonal rat biliary sIgA also induced PMN locomotor response (Fig. 2), and this migration was again of the chemotactic type (data not shown).

IgG1 and IgG2b also induced a dose-dependent locomotor response of PMN (Fig. 3). However, in contrast to IgA, they showed a chemokinetic type of activity. Even after IgG1 or IgG2b was added to the upper chamber, the degree of migration was not reduced (Table 2). On the other hand, IgG2a induced maximal locomotion at a concentration of 0.3 mg/ml, and at higher concentrations locomotion was decreased (Fig. 3). Furthermore, this subclass showed a chemotactic activity (Table 2).

Effect of IgA on FMLP-induced chemotaxis. The effect of IgA on the chemotactic movement induced by FMLP was investigated. The polymeric IgA preparation was added to the lower or upper chamber in order to make a positive or negative gradient of IgA concentration between the two chambers (Fig. 1a and b, respectively). When polymeric IgA (0.3 mg/ml) was added to the cell suspension in the upper chamber (a negative gradient of IgA) and the migration of PMN towards FMLP alone in the lower chamber was assessed, a leftward shift of the dose-response curve of FMLP-induced chemotaxis was observed (Fig. 1a). Namely, chemotaxis of PMN in 0.3 mg of IgA per ml towards suboptimal concentrations of FMLP without IgA was significantly enhanced, and now 3×10^{-8} M FMLP induced maximal chemotaxis. On the other hand, chemotaxis of PMN in 0.3 mg of IgA per ml towards the previously optimal (10^{-7} M) and higher concentrations of FMLP without IgA was significantly inhibited.

When polymeric IgA (0.3 mg/ml) was added only to the lower chamber with FMLP (positive gradient of IgA), PMN chemotaxis towards every concentration of FMLP tested (except for two concentrations, 10^{-7} and 3×10^{-8} M) was significantly enhanced by IgA (Fig. 1b). At an FMLP concentration of 3×10^{-9} M, the degree of enhancement was about sixfold higher than the sum of migrations induced by IgA and FMLP separately.

Locomotion induced by immune complexes. Soluble immune complexes containing IgG1 or IgG2b induced higher locomotor responses of PMN than antibody alone ($P < 0.01$) (Table 3). These responses were also of the chemokinetic type (Table 3). However, immune complexes containing monomeric IgA, polymeric IgA, or IgG2a in antigen excess induced a slightly lower response than antibody alone, although this was not statistically significant (Table 3). These

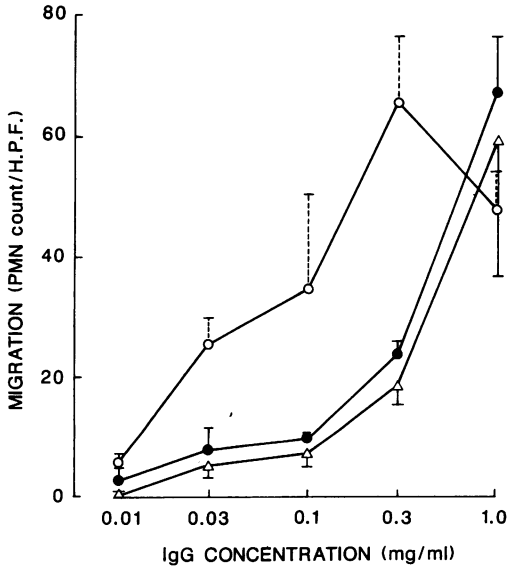

FIG. 3. PMN migration induced by IgG subclasses. Degrees of migration are plotted against the concentration of IgG1 (●), IgG2a (○), and IgG2b (△). Each point represents the mean ± SD (for IgG1, $n = 3$; for IgG2a and IgG2b, $n = 6$).

TABLE 3. Migration induced by antibodies alone and by immune complexes[a]

Antibody	Mean PMN count/H.P.F. ± SD (range) with:	
	Antibody alone[b]	Antibody complexed with DNP_8-BSA[c]
Monomeric IgA	14.3 ± 4.5 (9.8–22.4)	6.9 ± 1.5 (5.0–8.4)
Polymeric IgA	39.7 ± 13.7 (27.6–63.1)	39.5 ± 12.4 (22.7–55.8)
IgG1	20.3 ± 13.3 (7.5–33.3)	55.5 ± 19.0[d] (27.5–78.0)
		44.2 ± 0.6[e] (43.5–44.5)
IgG2a	45.6 ± 6.4 (37.1–53.4)	38.1 ± 6.4 (29.3–42.6)
IgG2b	9.9 ± 3.9 (5.9–17.6)	40.4 ± 9.1[d] (23.0–55.5)
		32.8 ± 5.7[e] (26.5–37.5)

[a] See Table 1, footnote *a* ($n = 6$).
[b] Concentration, 0.1 mg/ml.
[c] Concentration, 0.1 mg of antibody per ml with 0.13 mg of DNP-BSA per ml (antigen excess).
[d] $P < 0.01$ versus antibody alone.
[e] Same concentrations of immune complexes were also added to the cell suspension in the upper chamber ($n = 3$).

results did not seem to be a "deactivation" due to high concentrations of agonist often observed in the locomotor responses of PMN, because more dilute polymeric IgA- or IgG2a-immune complexes (final concentration of antibody, 0.03 mg/ml) did not enhance the migration either (data not shown). DNP_8-BSA by itself did not induce stronger migration than the negative control (HSA/HBSS) (data not shown).

DISCUSSION

The results of the present study show that both polymeric and monomeric IgAs from rats have a dose-dependent chemotactic activity for rat PMN. Monomeric IgG also induced a locomotor response of PMN; however, the nature of the induced locomotion differed between IgG subclasses. IgG1 and IgG2b induced a chemokinetic type of movement, since no concentration gradient was required, as shown in Table 2. This locomotor effect was dose dependent (Fig. 3). In contrast, IgG2a had a chemotactic activity and induced maximal migration at a relatively low concentration (0.3 mg/ml). Furthermore, in the form of soluble immune complexes, IgG1 and IgG2b induced stronger migration than antibody alone, but IgA and IgG2a did not. Polymeric IgA was found to enhance (leftward shift of the dose-response curve) FMLP-induced chemotaxis. However, migration of PMN at optimal and higher FMLP concentrations was significantly suppressed, as noted by other investigators (14, 24, 25).

Immunoglobulins have a recognized role as phlogistic agents in the inflammatory process. Part of this role has been considered to be due to the ability of immune complexes to induce PMN migration through activation of the complement cascade, with liberation of the chemotactic factor C5a. In fact, scattered reports (15, 26) suggest that aggregated or nonaggregated immunoglobulins induced PMN chemotaxis only in the presence of complement. However, in these assay systems, the PMN were suspended in a solution containing serum. Therefore, a considerable amount of immunoglobulins were also present in the upper chamber, and evaluation of the activity of immunoglobulins in inducing PMN migration would have been impossible.

Several authors have addressed the effects of immunoglobulin molecules as native proteins and aggregates or immune complexes on PMN motion. However, the data are in conflict. Some studies suggest that in serum-free system, IgG has no effect on PMN migration (28). However, the IgG used in these studies was a commercial preparation which contained aggregates. A prior study done with IgG freshly prepared from human serum found IgG to be chemotactic at a low concentration (22). By contrast, IgG aggregates (13) and immune complexes (29) can stimulate increased PMN adhesion. If these aggregates are bound to chemotaxis membranes, they will reduce PMN chemotaxis to other stimulants (13). The present study revealed clearly that rat immunoglobulins could induce the locomotor response of PMN in the absence of complement. Moreover, the nature of the migration induced by IgA and IgG subclasses, as described above, was clearly delineated. Although binding of immunoglobulin to the chemotaxis membrane could have influenced these data, the short incubation time (20 min) and the presence of albumin (5 mg/ml) in all buffer solutions made significant immunoglobulin binding to the polycarbonate membrane unlikely.

Van Epps et al. (24, 25) demonstrated that polymeric IgA *from myeloma* patients inhibited the chemotactic movement of human PMN towards various chemoattractants. Kemp et al. (14) confirmed this phenomenon, using polyclonal IgA isolated from human serum. In these studies, IgA was added mainly to the cell suspension (in the former report) or to both upper and lower chambers (in the latter). However, the ability of IgA by itself to induce PMN migration was not taken into consideration. Moreover, their studies were limited to maximally stimulated chemotaxis. Thus, although their results were partially consistent with ours (i.e., in our studies, IgA in the upper chamber inhibited migration towards the optimal concentration of FMLP), the ability of IgA to enhance migration, clearly shown in our report, was not revealed.

Antigen itself was shown in vitro to be chemotactic for PMN when they were coated (actively or passively) with antigen-specific cytophilic antibody (12). The mechanism of this effect, however, remains unexplained, as it required the presence, together with the PMN, of small amounts (0.5%) of normal serum, which contains both complement and immunoglobulins.

It is of interest that, although IgG1 and IgG2b, which were chemokinetic, induced stronger migration in the form of immune complexes, IgA and IgG2a, which were chemotactic, did not. As these locomotor responses seem to be mediated by Fc receptors for human immunoglobins on human PMN (22), differences of properties (affinity?) between Fc receptors for each immunoglobulin, which remain to be elucidated, might explain this phenomenon. About 80% of human PMN express Fcγ receptors, but only $\pm$ 35% express Fcα receptors (6). Heterogeneity among human PMN (8) was also demonstrated in chemotaxis towards different chemotactic agents (10) and in oxidative metabolism (3). It is therefore possible that rat PMN are also heterogeneous. Certainly, our observed differences for the subclasses of IgG suggest that this is so. We are presently studying their Fc receptor heterogeneity.

Although we have clearly demonstrated the above findings with the use of defined antibody populations, the biological implications of these data are uncertain. Moreover, our data should be limited to the specific in vitro conditions used in the present experiments. As IgG1 and IgG2b, two major IgG subclasses of rat serum, are chemokinetic, their in vivo role may occur only after immune complex formation, when the enhanced chemokinetic activity of these IgG subclasses for PMN might be important. On the other hand, the ability of IgA to attract PMN (chemotactic) might be related to the fact that IgA is mainly present on mucosal surfaces, where PMN are rare. Although the chemotactic potency of IgA seems to be weak, it can exhibit significant synergy with other chemoattractants, as described above (Fig. 1b). Conceivably, IgA could modify the chemotactic movement of PMN induced by other chemoattractants, favoring mobilization of PMN from the circulation to the locus of bacterial infection on the mucosal surface, where IgA is abundant and complement is not (19).

It will be of interest to investigate whether, in addition to their effect on PMN motility, insolubilized rat IgA and IgG or their insoluble immune complexes will, as observed for humans (2, 11), bind to rat PMN and induce degranulation and lysozomal enzyme release. Furthermore, since mouse IgA-immune complexes apparently enhanced oxygen radical release by rat pulmonary alveolar macrophages but not by rat PMN, with possible pathogenetic consequences in the lung (27), the influence of rat IgA-immune complexes on oxygen metabolite production by rat PMN should also be investigated to obtain a coordinate picture of the reciprocal

influences of rat IgA- and IgG-immune complexes on rat PMN and macrophage functions in immune defense and injury. Our group is actively engaged in such studies.

In conclusion, rat immunoglobulins and immune complexes could induce locomotor responses of PMN in vitro at physiological levels in the absence of complement. The nature of the induced locomotion (chemotactic or chemokinetic) differed between classes and subclasses. Although monomeric and polymeric IgA and IgG2a were chemotactic and, in the form of soluble immune complexes, did not show higher chemotactic activity than antibody alone, IgG1 and IgG2b were chemokinetic and, in the form of immune complexes, induced a higher degree of migration than antibody alone. These results indicate that IgG1 and IgG2b might stimulate the mobility of PMN in the form of immune complexes and that IgA could modify the chemotactic movement induced by other chemotactic factors and thus facilitate accumulation of PMN into mucosal sites of infection.

ACKNOWLEDGMENTS

This work was supported by the Belgian State—Prime Minister's Office—Science Policy Programming (Concerted Actions grant 82/87-39).

The technical help of J. Naze-De Mets and A. Langendries is highly appreciated.

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