IgE-MEDIATED CHEMOTAXIS OF RAT BASOPHILIC LEUKEMIA CELLS TOWARDS SPECIFIC ANTIGEN*

BY NORMAN ORIDA,† JOSEPH D. FELDMAN, DAVID H. KATZ AND FU-TONG LIU§

From the Department of Immunology, Scripps Clinic and Research Foundation, and the Department of Immunology, Medical Biology Institute, La Jolla, California 92037

Although the accumulation of basophils at sites of inflammation is well documented (1, 2), little information exists on the exact attractant molecules involved in the directional response. A general hindrance to analyzing the mechanisms that underly basophilic infiltration, and chemotaxis in general, has been the lack of experimental systems in which attractant molecules and their receptors are well defined. Using monoclonal immunoglobulin E (IgE) and defined antigen, we have studied specific chemotactic responses of rat basophilic leukemic (RBL) cells. RBL cells have functional and biochemical characteristics similar to basophils and mast cells and have been used as model systems for the study of basophil and mast cell biochemistry and physiology (3). Like normal mast cells, RBL cells have the capacity to bind IgE through characteristic high affinity membrane receptors specific for the Fc region of the immunoglobulin (FcRe) (4). The membrane-bound IgE imparts a capability to the basophil to interact with antigen during activation that results in mediator production and elaboration (5, 6). Specificity in response to antigen challenge is defined by the specificity of the bound IgE. Herein, we present our findings on IgE-mediated chemotactic responses of RBL cells whose specificity is also dictated by the antigen specificity of the IgE bound by the FcRe.

Materials and Methods

Reagents. Dinitrophenyl (DNP)-specific monoclonal mouse IgE from hybridoma H1-DNP-e26.82 (α-DNP-IgE) was described previously (7). A ragweed fraction A (Rag; Miles Laboratories Inc., Elkhart, IN)-specific IgE-secreting hybridoma (α-Rag-IgE) was similarly constructed. DNP12-bovine serum albumin (DNP-BSA) was prepared as described (8), and rat myeloma proteins IgG1 (IR27), IgG2a (IR530), and IgE (IR162) from Lou/M/Ws1 rats were purified as described (9, 10). Dr. G. Boltz-Nitelescu (University of Vienna, Austria) donated rabbit anti-IR162 antiserum.

Cells. Sublines of RBL cells RBL-1, 926a, and 2H3-C were provided by Dr. C. Fewtrell and Dr. C. Isersky, National Institutes of Health. Subline 4A was cloned from existing RBL cell lines in this laboratory. All sublines were grown in 75-cm² tissue culture flasks in the presence of RPMI 1640 supplemented with 10% fetal calf serum 1% penicillin-streptomycin with fungizone and 0.1% L-glutamine ("medium"). Before each experiment, cells were harvested from culture flasks with trypsin-versene, washed twice with medium, and resuspended to 2–4

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† To whom correspondence should be addressed at the Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, CA 92037. Dr. Orida is a Postdoctoral Fellow of the Muscular Dystrophy Association.

§ Scholar of the Leukemia Society of America.
× 10⁶/ml in medium.

Chemotaxis and Serotonin Assays. The following were added to an aliquot of the harvested cell suspension: nothing, α-DNP-IgE, α-Rag-IgE, or IR162, (of unknown specificity), to a final concentration of 3 μg/ml. The cell suspension was incubated with gentle shaking for 1 h at 37°C. In two experiments, 926a cells were incubated for 0.5 h in the presence of 30 μg/ml of IR27 and 30 μg/ml of IR330. Then, α-DNP-IgE was added to the cell suspension without washing to a final concentration of 3 μg/ml and the incubation continued for an additional 1 h. After the incubation period, all cells were washed twice with 4 ml Gey’s balanced salt solution (GBSS) and resuspended with GBSS to a final concentration of 10⁶/ml. 100 μl of this cell suspension were placed in the upper well of blindwell Boyden chambers (Microfiltration Systems, Dublin, CA) (11). The lower well of the Boyden chamber contained GBSS with or without additions. Between the upper and lower chambers was a 10-μm-thick polycarbonate filter with a 5-μm pore size. After the chambers were incubated for 2 h at 37°C in a humidified incubator, the filters were removed, fixed, and stained with Wright-Giemsa. An assay measuring release of incorporated [³H]serotonin to assess RBL cell degranulation was performed as described previously (12).

Results and Discussion

IgE-mediated Chemotaxis of RBL Sublines. After sensitization with saturating doses of α-DNP-IgE, all sublines of RBL cells were tested for chemotaxis towards DNP-BSA. As shown in Fig. 1, the 926a subline was significantly positive for chemotaxis towards DNP-BSA. Subline 2H3-C was also positive, but to a much lesser degree, while two others, RBL-1 and 4A, were not. Cells from sublines 2H3-C and 926a underwent chemotaxis when the concentration of DNP-BSA in the lower well of the Boyden chamber was between 1 ng/ml and 1 μg/ml. Optimal responses were observed when 100 ng DNP-BSA/ml was used.

Chemotactic behaviors were further analyzed using subline 926a, the highest responder. As shown in Table I, the unhaptenated carrier, BSA, at 100 ng/ml did not elicit chemotactic responses (cf., row 2 vs. row 1). Negligible migration to the lower surface of the chemotaxis membrane occurred when 100 ng DNP-BSA/ml was in both upper and lower wells of the Boyden chamber, indicating that chemokinesis was not solely responsible for the cell migration (row 3). IgE with specificity for stimulating antigen was required because no chemotactic responses to DNP-BSA occurred when: (a) sensitization with α-DNP-IgE was omitted (rows 4–8) or (b) cells were sensitized with rat IgE of unknown specificity (IR162; rows 9–13). Furthermore, as shown in Fig. 2, when cells were sensitized with the monoclonal IgE, α-Rag-IgE, Rag did not elicit chemotaxis from cells sensitized with α-DNP-IgE, but did elicit chemotaxis from cells sensitized with α-Rag-IgE. Finally, 926a cells which were presensitized with rat IgG1 and IgG2a before sensitization with α-DNP-IgE did undergo chemotaxis toward DNP-BSA (Table I, row 14), indicating that the interaction of α-DNP-IgE with the cells was not interfered with by IgG, and was presumably specific between IgE and FcRe.

IgE-mediated Serotonin Release of RBL Sublines Does Not Parallel Chemotactic Behavior. Fig. 3 demonstrates that when sensitized with α-DNP-IgE, cells from subline RBL-1 did not release [³H]serotonin, but that cells from sublines 926a, 2H3-C, and 4A did, after challenge with DNP-BSA. (Note that subline 4A failed to display chemotactic responses under similar conditions of sensitization and challenge.) The threshold concentration for [³H]serotonin release was ~10 ng DNP-BSA/ml for sublines 2H3-C and 926a, and ~1 ng DNP-BSA/ml for subline 4A. Further analysis of the release response in subline 926a, the highest responder for chemotaxis, showed that specific IgE was required because [³H]serotonin was not released after DNP-BSA
Chemotaxis of RBL sublines sensitized with α-DNP-IgE towards specific antigen (DNP-BSA). Antigen concentrations are those in the lower well of the Boyden chamber. Chemotaxis was scored as the number of nuclei counted on the lower surface of the chemotaxis membrane and was totalled from 30 randomly selected high power microscope fields (X 970). Data are presented as means of at least three separate experiments. Duplicate counts of filters within each experiment varied by <10%.

**Table I**

Antigen Specificity Requirements for IgE-mediated Chemotaxis of Cells from Subline 926a

| Sensitization | Stimulus in lower well of Boyden chamber | Chemotaxis |
|---------------|----------------------------------------|------------|
| 1 α-DNP-IgE   | 100 ng DNP-BSA/ml | 179 ± 19 (14) |
| 2 α-DNP-IgE   | 100 ng BSA/ml | 1 ± 1 (8) |
| 3 α-DNP-IgE   | 100 ng DNP-BSA/ml, upper and lower wells | 5 ± 2 (6) |
| 4 None        | 1 ng DNP-BSA/ml | 3 ± 2 (2) |
| 5 None        | 10 ng DNP-BSA/ml | 5 ± 1 (2) |
| 6 None        | 100 ng DNP-BSA/ml | 5 ± 1 (2) |
| 7 None        | 1 μg DNP-BSA/ml | 4 ± 3 (2) |
| 8 None        | 10 μg DNP-BSA/ml | 2 ± 2 (2) |
| 9 IR162 IgE myeloma | 1 ng DNP-BSA/ml | 0 (1) |
| 10 IR162 IgE myeloma | 10 ng DNP-BSA/ml | 0 (1) |
| 11 IR162 IgE myeloma | 100 ng DNP-BSA/ml | 0 (1) |
| 12 IR162 IgE myeloma | 1 μg DNP-BSA/ml | 0 (1) |
| 13 IR162 IgE myeloma | 10 μg DNP-BSA/ml | 0 (1) |
| 14 IgG1 and IgG2a, then α-DNP-IgE | 100 ng DNP-BSA/ml | 178 ± 11 (2) |

Chemotaxis was scored as the number of nuclei counted on the lower surface of the chemotaxis membrane and totalled from 30 randomly selected high power microscope fields (X 970). Data are presented as mean ± 1 SEM. Numbers in parentheses represent numbers of experiments performed.

In this study, we have demonstrated IgE-mediated chemotactic responses by RBL cells towards specific antigen. To our knowledge, this is the first report of chemotaxis by a basophil-like cell requiring IgE and mediated via FcεR. Although chemotaxis by basophils towards complement factors (13) and supernatants from mitogen-stimulated lymphocyte cultures (14) has been reported, our results were obtained using an experimental system in which both stimulant and receptor molecules are well defined.
Chemotaxis toward DNP-BSA by 926a cells is a sensitive response and will take place when the concentration of the stimulating molecules is on the order of $10^{-10}$ M (10 ng DNP-BSA/ml), a molar concentration similar to that of formyl peptides required to initiate chemotaxis of human neutrophils (15, 16).

It is pertinent to note that chemotaxis and serotonin release were not sustained in parallel throughout the range of antigen concentrations tested in sublines 2H3-C and 926a in which both properties were present (Figs. 1 and 3). Loss of chemotactic responses at higher doses of DNP-BSA for subline 926a, for example, probably reflects the diminished gradient effect of antigen in the Boyden chamber at such higher doses. However, subline 4A, a strong serotonin releaser (Fig. 3), showed no chemotactic behaviors (Fig. 1). These findings suggest that chemotaxis and secretion can be distinct unlinked processes that occur during basophil activation. It remains to be seen how the basophil segregates these two functions which are both initiated via FcRε. An approach to this problem might be to reconstitute chemotactic and secretory responses by genetic complementation through cell fusion using responder and nonresponder RBL sublines (17).

The findings from our study of RBL chemotaxis in vitro predict that IgE bound to membrane FcRε could direct specific in vivo chemotactic responses to inflammatory stimuli by basophils and possibly other cells bearing FcRε, such as macrophages (10, 18) and T lymphocytes (19). A similar proposition has been made in regard to the
function of surface Ig on B lymphocytes (20). Further studies should ultimately verify such roles for this important immunoglobulin and its receptor.

Summary

We evaluated chemotactic properties of four sublines of rat basophilic leukemia cells using blindwell Boyden chamber assays. After sensitization with a mouse monoclonal IgE directed against dinitrophenyl (DNP), cells from sublines 2H3-C and 926a underwent chemotaxis toward DNP-bovine serum albumin (BSA) and sublines RBL-1 and 4A did not. Chemotactic responses required specific IgE and were determined by the IgE antigen specificity used for sensitization. The threshold for chemotaxis was on the order of $10^{-10}$ M DNP-BSA. Release of incorporated $[^3H]$-serotonin did not always parallel chemotactic responses, which suggests that chemotaxis and secretion may be two unlinked processes that occur during basophil activation. Our results predict a possible in vivo mechanism whereby specific chemotactic responses of basophils and other FcRε-bearing cells are mediated via specific IgE bound to membrane FcRε.

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