T CELLS PRODUCE AN ANTIGEN-BINDING FACTOR WITH IN VIVO ACTIVITY ANALOGOUS TO IgE ANTIBODY*

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Delayed-type hypersensitivity skin reactions are mediated by recirculating, sensitized Ly-1$^+$ T cells (1, 2) that enter the tissues and are activated by specific antigen. This leads to the release of various nonspecific macromolecular mediators (lymphokines) that attract various leukocytes to leave the circulation and enter the tissues to comprise a characteristic infiltrate of inflammatory cells. In mice, this cellular recruitment requires that the activated T cells also activate resident tissue mast cells to release the vasoactive amine serotonin, which causes gaps to form between vascular endothelial cells (3–6). This allows the leukocytes to emigrate into the extravascular tissue spaces in response to chemattractant lymphokines (5, 6).

The fact that T cell-dependent activation of mast cells is required for elicitation of delayed-type hypersensitivity led us to investigate whether a T cell product could mimic some of the functions of immunoglobulin E (IgE) antibody. We have described (7) a T cell-derived antigen-binding factor that transfers an immediate hypersensitivity-like reaction. In the present study this T cell factor was compared with a hybridoma IgE antibody. Both transferred sensitization for elicitation of immediate-type skin reactions with accompanying vascular permeability. Neither was active in mast cell-deficient mice. The T cell factor was distinguished from IgE by a number of immunochemical and biological properties; including affinity chromatography using specific anti-IgE and anti-factor antibodies, and a shorter duration of passive sensitization. The T cell factor is a suitable candidate for participation in the mechanism by which T cells activate mast cells in delayed-type hypersensitivity.

Materials and Methods

Mice. Male CBA/J and two types of mast cell-deficient mice, [WBB6F$_1$ (WB-W/+ × C57Bl6-W^v/+)-W/W^v and their normal litter mates (WBB6F$_1$-+/+), and WCB6F$_1$ (WC-Sl/+ × C57Bl/6-Sl^d/+)-Sl/Sl^d and their normal litter mates (WCB6F$_1$ − +/+)] were obtained from The Jackson Laboratory, Bar Harbor, ME and were rested at least 1 wk in an air-filtered enclosure before use.

Reagents. Picryl chloride (PCL)[1] (Chemotronix Inc., Swannonoa, NC) recrystalized three times from methanol/H_2O before use, and oxazolone (OX) (Gallard-Schesinger Chemical Mfg.

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[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; DNP, 2,4-dinitrophenyl; EACA, epsilon amino caproic acid; ELISA, enzyme-linked immunosorbent assay; OX, oxazolone; OXF, oxazolone factor; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; PCL, picryl chloride; PCLF, picryl chloride factor; TNP, 2,4,6-trinitrophenyl.

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Corp., Carle Place, NY), recrystalized three times from ethanol, were protected from light in a dessicator during storage at 25°C. Bovine gamma globulin (BGG) and epsilon-amino caproic acid (EACA) were purchased from Sigma Chemical Co., St. Louis, MO. Ascites fluid containing 2 mg/ml of murine monoclonal anti-2, 4-dinitrophenyl (DNP) IgE antibody (H1 DNP-ε-26), hapten affinity-purified monoclonal anti-DNP IgE antibody prepared from this ascites, and monospecific rabbit anti-IgE made with this monoclonal IgE were gifts from Dr. David H. Katz and Dr. Fu-Tong Liu, Dept. of Immunology, Medical Biology Institute, La Jolla, CA (8). Another anti-DNP monoclonal IgE (SPE-7-IF, both in ascites fluid and hapten affinity purified) was provided by Dr. Zelig Eschar, Weizmann Institute, Rehovot, Israel (9). Ascites fluid containing anti-ovalbumin monoclonal IgE was provided by Dr. Irmgard Böttcher (10).

General Methods. Affinity chromatography was performed at 4°C with Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) as the support matrix. Proteins were covalently attached to sepharose by the cyanogen bromide technique (11). 2,4,6-trinitrophenyl (TNP) was covalently attached to BGG-Sepharose by reaction with trinitrobenzene sulfonic acid (Eastman Laboratory and Specialty Chemicals, Eastman Kodak Co., Rochester, NY) in 0.2 M Na_2CO_3 at pH 11. A ratio of 10 mg of trinitrobenzene sulfonic acid for each 10 mg of BGG was used. OX coupling to BGG was performed by reacting OX dissolved in ethanol with BGG sepharose so that 10% of the reaction mixture was ethanol in 0.2 M Na_2CO_3 at pH 11. TNP-EACA was prepared by reaction of trinitrobenzene sulfonic acid with EACA in 0.2 M Na_2CO_3 at pH 11. TNP-EACA was extracted from the acidified (pH $\leq$ 2.5) reaction mixture with ethyl acetate and crystalized from methyl alcohol/H_2O.

Contact Sensitization and Production of Specific Antigen-binding Factors from Lymphoid Cells of Contact-sensitized Mice. Donor CBA/J male mice (8–10 wk old) were sensitized by topical application of either 0.15 ml of 5% PCL or 3% OX in absolute ethanol and acetone (3:1) to the shaved abdomen, chest, and four feet. 4 d later spleen and peripheral lymph node cells from these mice ($\sim$1–2 $\times$ 10^8 cells/mouse) were cultured in vertical 250-ml flasks (70–150 ml/flask) at 1.5 $\times$ 10^7 cells/ml in serum-free RPMI 1640 medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with mycostatin, gentamycin, and streptomycin, for 48 h at 37°C in 5% CO_2. The culture fluid supernatant was then separated from the cells by centrifugation for 7 min at 25°C at 2,500 rpm in an HG 4L rotor (DuPont Instruments-Sorvall Biomedical Div., Wilmington, DE) and antigen-binding material (hereafter called PCL factor, PCLF, or OX factor, OXF) was purified by addition of the culture fluids (100–200 ml) to an affinity matrix composed respectively of TNP-BGG-linked Sepharose beads or OX-BGG-linked Sepharose beads, followed by overnight incubation of the mixture at 4°C with gentle stirring. The mixtures were then poured into columns over a bed of G25 Sephadex (Pharmacia Fine Chemicals), and washed with 5 column vol of phosphate-buffered saline (PBS). The TNP-BGG-Sepharose columns that bound material from supernatants of cells from mice sensitized with PCL were then eluted at 4°C with 0.05 M TNP-EACA. In some experiments, 5 M guanidine was also used for elution. The OX-BGG Sepharose columns that bound material from supernatants of cells from mice sensitized with OX were eluted with 5 M guanidine. The respective eluates, in a volume of $\sim$5–10% of the original supernatants, were then dialyzed exhaustively at 4°C against PBS. Finally, PCLF and OXF were dialyzed into 0.15 M NaCl for intravenous transfer. The protein content of purified PCLF ranged from 0.1–0.3 mg/ml for the 12 preparations used in this study, as measured by the method of Bradford (12). In a typical experiment supernatant obtained from lymphoid cells of 50–100 PLC-sensitized mice resulted in 2-mg yield of hapten affinity-purified PCLF protein, and supernatant obtained from lymphoid cells of 30 OX-sensitized mice resulted in 2.4-mg yield of hapten affinity-purified OXF protein.

Bioassay of PCLF or OXF. Unless otherwise stated, 75 μg PCLF or OXF in 0.5 ml 0.15 M NaCl was injected intravenously via the retro-orbital plexus into lightly ether-anesthesized mice and both sides of their ears were challenged immediately thereafter by topical application of a drop (27-gauge needle) of 0.8% PCL or 0.8% OX in olive oil. Duplicate measurement of ear thickness was made with an engineer's micrometer (Mitutoyo, Tokyo, Japan) before challenge and at various times from 0.5–24 h later. The increment in ear thickness was expressed as the mean $\pm$ SE in units of 10^{-3} cm (13). In each experiment the ears of a separate group of

noninjected controls were challenged and measured similarly to mice that received either PCLF or OXF.

Direct Binding Enzyme-linked Immunosorbent Assay (ELISA) for Various Antigenic Determinants in PCLF or OXF. Each of the 12 different preparations of PCLF used in this study, a preparation of OXF, and mouse Ig standards were coated onto the wells of plastic microtrays at a concentration of 100 ng/well. As described previously (7), none of the PCLF preparations, nor the OXF preparation, had Ig antigenic determinants detected by anti-κ, -λ, -μ, -α, -γ, -δ, or -ϵ antisera, while all factor preparations had antigenic determinants detected by three different rabbit antisera prepared against antigen-specific T cell factors from other systems (7, 14–16).

Production of Rabbit Anti-factor Antisera. Antisera were produced in collaboration with Dr. Robert Cone (Dept. of Pathology, Yale University School of Medicine, New Haven, CT) according to the method used to produce rabbit anti-T cell derived suppressor factor (14, 15, 17). Rabbits were injected with 200 μg PCLF emulsified in complete Freund's adjuvant and were boosted with PCLF and poly AU (14, 15). Antisera obtained were routinely screened for anti-mouse Ig heavy and/or light chain isotypes and anti-hapten activity by ELISA. Anti-PCLF sera used were negative for binding to Ig determinants, but always contained anti-TNP activity. This anti-TNP activity was absorbed out by passage over TNP-BGG Sepharose absorbents, and this absorbtion was confirmed by ELISA using TNP-BGG-coated plates.

Competitive Binding Radioimmunoassay of IgE. A modified solid-phase radioimmunoassay was used (18). The 18% Na_2SO_4 globulin fraction of rabbit anti-IgE (1:1,000 dilution) was coated onto polystyrene flat-bottomed nonsterile microtrays (Linbro Scientific, Hamden, CT). Hapten affinity purified monoclonal IgE (H1 DNP-ϵ-26) was iodinated with ^{125}I by the lactoperoxidase method (19). About 15 ng of ^{125}I-IgE (75,000 cpm) in PBS containing 0.05% triton-X 100 (New England Nuclear, Boston, MA) were mixed with various concentration of either two different preparations of PCLF, two different anti-DNP hybridoma IgE antibodies or anti-ovalbumin hybridoma IgE antibody, and then, were added to the anti-IgE-coated wells and incubated at 20°C temperature for 12 h after which the wells (in duplicate) were washed, dried, excised, and placed in tubes for counting in a gamma counter. The results were expressed as the percent of ^{125}I-IgE counts added per well that bound to the well surface.

Affinity Chromatography with Anti-IgE vs. Anti-PCLF Sepharose. An 18% Na_2SO_4 precipitate of rabbit anti-IgE and rabbit anti-PCLF sera was prepared, and the globulin fractions were resolubilized and conjugated separately to Sepharose via cyanogen bromide (11). Duplicate 1.0-ml columns of each type were prepared, and IgE (H1 DNP-ϵ-26 ascites) and PCLF were applied to each column at 4°C. The material passing through the column (the filtrate) was collected and the columns were washed with PBS and then eluted with 0.2 M Na_2CO_3 (pH 11). The resulting eluate was dialyzed against PBS and then against saline. The filtrate and the eluate from each column were then transferred intravenously to normal recipients that were challenged immediately thereafter by ear painting with 0.8% PCL in olive oil.

Assay of Increased Vascular Permeability. Bovine serum albumin was iodinated with ^{125}I by the lactoperoxidase method (18). Iodinated albumin was mixed with PCLF or IgE that were transferred intravenously to separate groups of mice that were then challenged immediately by painting their ears with 0.8% PCL. 4 h later, after ear thickness measurements were repeated, the animals (and controls that just received ^{125}I-albumin before PCL challenge) were killed and their ears were excised and placed in tubes for counting in a gamma counter.

Affinity Chromatography of Polyclonal IgE Antibody Activity Determined by Passive Cutaneous Anaphylaxis (PCA) Titration. Sera containing polyclonal IgE antibody from BALB/c mice that were primed and boosted multiple times with DNP conjugated ascaris antigen were provided by Dr. David H. Katz. PCA titratious were performed by making an initial 1:50 dilution and subsequent twofold dilutions in Locke's buffer containing 0.1% bovine serum albumin and injecting 50 μl of the various dilutions into the flank skin of three Sprague Dawley rats (150 g). 2 h later, 1 mg of DNP-albumin was injected intravenously with 1% Evan's blue dye, and 30 min later the animals were killed and the last dilution resulting in a blue spot of extravasated dye of at least 5-mm diam in at least two rats was taken as the PCA titer. The original hyperimmune serum had a PCA titer of ~1:40,000 since titration of the 1:50 dilution revealed a PCA titer of 1:800. The 1:50 dilution was passed through small affinity columns composed

of Sepharose coupled with either anti-IgE, anti-PCLF, or normal rabbit serum and the PCA titer of the material passing through the columns was determined as described above.

Results

Immune Cells Release Antigen-binding Factors that Can Transfer an Antigen-specific Immediate Hypersensitivity-like Reaction (Fig. 1). Cultured lymphoid cells from mice immunized by contact sensitization with either PCL or OX released supernatant factors that were specifically purified and concentrated by hapten affinity chromatography on columns coated with homologous hapten; i.e., TNP-BGG-sepharose and OX-BGG-sepharose, respectively. Intravenous transfer of these factors (PCLF and OXF) to normal nonimmune mice resulted in the ability to elicit a 4-h ear swelling response to challenge by the homologous contactant, but not by the other contactant (Fig. 1). Thus PCLF and OXF transferred the ability to elicit antigen specific immediate hypersensitivity-like reactions.

PCLF Transfers an Immediate Hypersensitivity Reaction Like IgE, but Does Not Compete with IgE in a Radioimmunoassay (Figs. 2, 3, and 4). The kinetics of ear swelling responses were similar after PCL challenge of mice that received either PCLF or IgE (Fig. 2). Both responses were evident within 30 min, peaked at 2–4 h, and rapidly declined thereafter (Fig. 2). Also, both responses were accompanied by significantly increased vascular permeability (Fig. 3). Despite the fact that PCLF could passively sensitize mice for elicitation of skin reactions of similar kinetics and vascular permeability as occurred in reactions mediated by IgE, there was no ability of PCLF to compete with ^{125}I-labeled IgE for binding to specific rabbit anti-mouse IgE (Fig. 4). In contrast, three different IgE hybridoma antibodies were strongly inhibitory (Fig. 4). Thus,

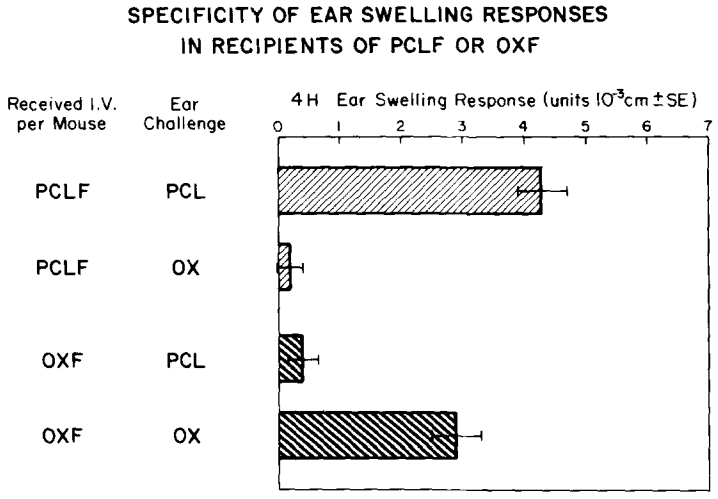

FIG. 1. Specificity of ear swelling responses in mice receiving PCLF or OXF. Lymph node and spleen cells from mice contact sensitized with PCL or OX were cultured for 48 h and supernatants were applied respectively to TNP-BGG or OX-BGG-Sepharose and PCLF and OXF eluted (see Materials and Methods). Separate groups of five CBA/J mice were injected intravenously with 75 μg PCLF or OXF. Immediately thereafter mice were challenged on the right ear with 0.8% PCL in olive oil and on the left ear with 0.8% OX. Ear swelling responses were measured with a micrometer. Five noninjected controls were challenged similarly and their resulting ear swelling response to PCL and OX was subtracted from the responses of the mice that were injected intravenously.

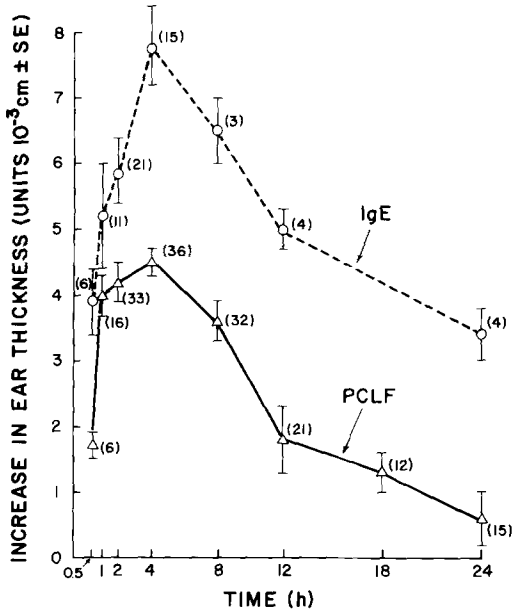

FIG. 2. Time course of ear swelling response in mice receiving PCLF or IgE. Separate groups of male CBA/J mice were injected intravenously with 75 μg PCLF (△) or with 25–75 μg IgE antibody (○). Immediately thereafter mice were challenged on both ears with 0.8% PCL in olive oil and then ear swelling responses were measured with a micrometer. Shown is the time course of the ear swelling response measured with a micrometer in 11 different experiments that used nine different PCLF preparations in three to six mice per group, and 5 different experiments that used IgE in three to six mice per group. In each experiment a group of noninjected controls was challenged similarly and their resulting ear swelling response at each time point (Fig. 1 in ref. 7) was subtracted from the response of the mice that were injected intravenously. The total number of mice used to calculate the mean ± SE is given in parenthesis at each time point.

PCLF had biologic activity analogous to IgE but had no ϵ antigenic determinants as detected by this sensitive competitive binding radioimmunoassay.

Passive Sensitization with PCLF is of Brief Duration Compared with IgE (Fig. 5). A biologic characteristic of IgE is prolonged passive sensitization. Experiments were performed to compare PCLF with IgE antibody for duration of passive sensitization. A reduced dose of IgE (6 μg) was transferred to produce immediate ear swelling responses equivalent to recipients of PCLF, when both groups were challenged within 30 min of transfer. Fig. 5 shows that equivalent ear swelling responses could be elicited in recipients of IgE either 0.5 or 48 h after intravenous transfer. In contrast, equivalent ear swelling responses were elicited in recipients of PCLF that were challenged at 0.5 h, but no response was elicited 48 h after intravenous transfer. Thus, IgE mediated prolonged passive sensitization, whereas passive sensitization with PCLF was of brief duration.

Affinity Columns Conjugated with Specific Antibodies Show that PCLF and IgE Are Distinct Entities (Fig. 6). PCLF transferred reactions that resemble those mediated by IgE, but no ϵ heavy chain determinants were detected in PCLF using either a direct-binding ELISA (7) or a competitive-binding radioimmunoassay (Fig. 4). Experiments with specific affinity columns were performed to further rule out the possibility that IgE-type molecules were responsible for the biologic activity of PCLF. Affinity

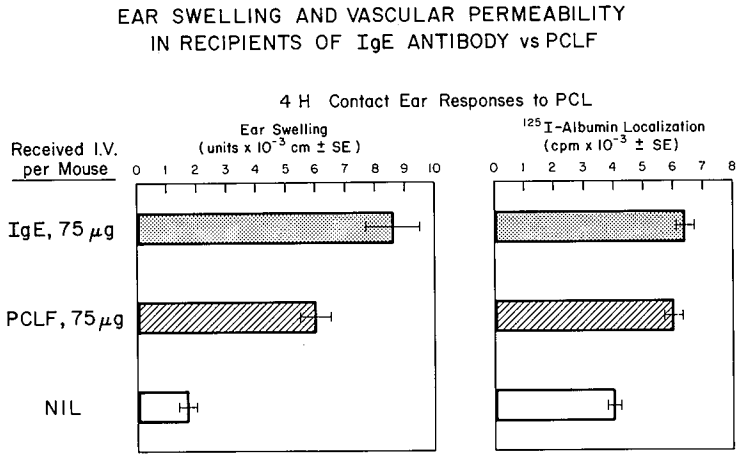

FIG. 3. Ear swelling and increased vascular permeability responses occur in recipients of IgE antibody or PCLF. Separate groups of four mice were injected intravenously with 75 μg IgE or PCLF or nothing and also received, intravenously, ^{125}I-bovine serum albumin. Immediately thereafter mice were challenged on both ears with 0.8% PCL and 4 h later ear swelling responses were measured with a micrometer. Ears were then excised and radioactivity was quantitated in a gamma counter.

columns consisted of Sepharose conjugated with rabbit anti-IgE or rabbit anti-PCLF. Fig. 6 shows that PCLF activity passed through the anti-IgE column, which retained IgE activity and from which IgE activity could be eluted. In contrast, PCLF was not retained by the anti-IgE column but was retained by, and could be eluted from, the anti-PCLF column.

The anti-IgE linked to the affinity column was induced by immunization of rabbits with the anti-DNP IgE hybridoma antibody that was used for the transfers (8). In the competitive binding radioimmunoassay, anti-IgE bound this IgE better than two other IgE hybridoma molecules (Fig. 4). Thus, it was possible that the ability of the anti-IgE column to remove hybridoma IgE activity was due to binding of a particular IgE clonotype, and thus was possibly due to antiidiotype or anti-IgE subclass activity. In addition, the ability of the anti-PCLF column to remove PCLF and not IgE hybridoma activity could be due to an IgE subclass specificity. To rule this out, diluted hyperimmune antisera containing polyclonal IgE antibody with a PCA titer of 1:800 was passed through the anti-IgE and anti-PCLF affinity columns and a column coated with normal rabbit serum. Passage through the anti-IgE column removed all detectable PCA activity, whereas passage through the anti-PCLF or normal rabbit serum columns did not affect the PCA titer (data not shown). Thus, polyclonal IgE activity was removed by the anti-IgE affinity column and was not removed by the anti-PCLF column.

Neither PCLF nor IgE Can Passively Sensitize Mast Cell-deficient Mice (Fig. 7). The ability of IgE to passively sensitize for elicitation of immediate hypersensitivity is dependent on its ability to bind to skin mast cells. Two different kinds of mast cell deficient mice were used to test whether the biologic activity of PCLF was also mast cell dependent. W/W^v mice have a defect in bone marrow precursors of skin mast cells and Sl/Sl^d mice have normal bone marrow precursors of skin mast cells, but lack cutaneous microenvironmental factors required for differentiation of the precursors

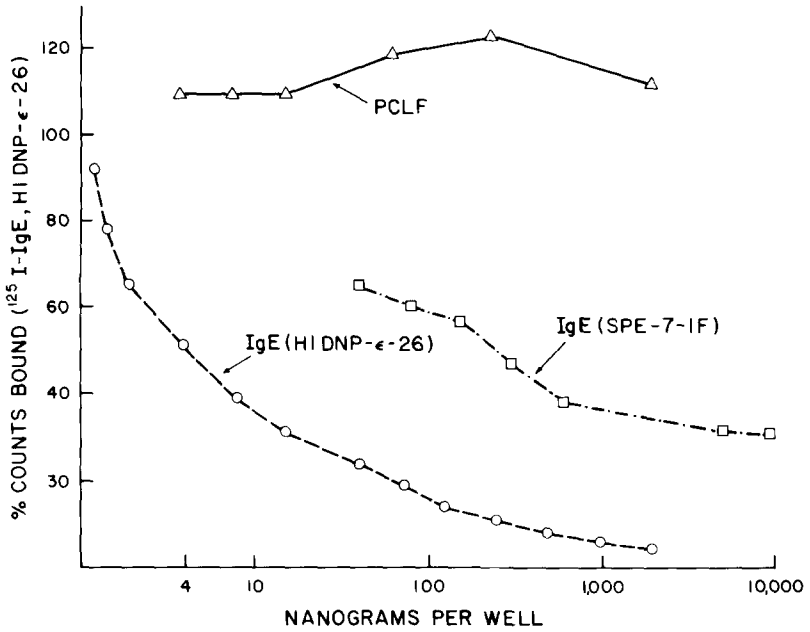

FIG. 4. Competitive binding radioimmunoassay of antigenic determinants in IgE. The IgG fraction (1:1,000 dilution) of rabbit anti-IgE hybridoma antibody (H1 DNP-ε-26) was coated onto wells of polystyrene microtrays. Then hapten affinity-purified ^{125}I-IgE (H1 DNP-ε-26) was mixed with increasing concentrations of either PCLF (△) (one preparation is shown, but two different preparations were assayed and gave similar results), the IgE (H1 DNP-ε-26) (○) that was used to induce the anti-IgE, or another anti-DNP hybridoma IgE (SPE-7-1F) (□). After incubation and washing, separate duplicate wells were cut out, placed in tubes for counting in a gamma counter, and counts bound were compared with counts added per well. A third hybridoma IgE antibody with ovalbumin specificity (10) was also assayed and gave results similar to IgE SPE-7-1F (results not shown).

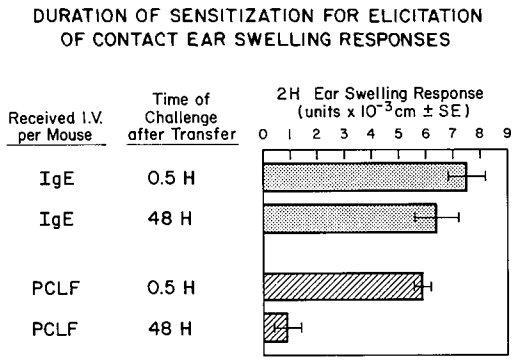

FIG. 5. Passive sensitization with PCLF is of brief duration compared with IgE. Separate groups of four mice were injected intravenously with 6 μg IgE or 100 μg PCLF. 30 min or 48 h later mice were challenged on both ears with 0.8% PCL, and 2 h later ear swelling responses were measured with a micrometer. A group of noninjected controls was challenged similarly and their resulting ear swelling response was subtracted from the response of the mice that were injected intravenously.

into mature mast cells (20–23). Both strains have ≤1% the numbers of cutaneous mast cells compared with their appropriate +/+ controls (20–23). Fig. 7 shows that PCLF, like IgE, was unable to passively sensitize either type of mast cell-deficient

AFFINITY COLUMNS AND TRANSFER OF EAR SWELLING RESPONSES

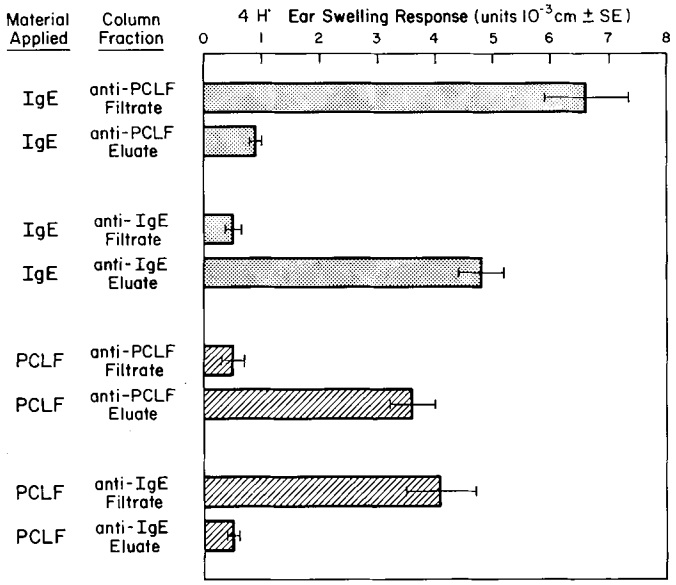

FIG. 6. Affinity column separation of IgE vs. PCLF activity that transfers the ability to elicit ear swelling responses. Two aliquots of IgE or PCLF were each applied to affinity columns coupled with anti-IgE or anti-PCLF. After the material passing through the columns (the filtrate) was collected, and the columns were washed thoroughly, material bound to the columns (the eluate) was obtained by passing 0.2 M Na_2CO_3 through the columns. After dialysis the filtrate and eluate were injected intravenously into separate groups of mice that were challenged immediately thereafter with 0.8% PCL. 4 h later ear swelling responses were measured with a micrometer. A group of noninjected controls was challenged similarly and their resulting ear swelling response was subtracted from the response of the mice that were injected intravenously.

mouse for elicitation of immediate hypersensitivity-like responses compared to +/+ littermates. Thus, the biologic activity of PCLF, like IgE, is mast cell dependent.

Discussion

We have shown that PCLF, a T cell-derived antigen-binding factor, has many in vivo functional characteristics analogous to IgE antibody. Both mediate antigen-specific skin swelling responses that are evident within 30 min and peak between 2 and 4 h after local challenge. Both responses are accompanied by increased vascular permeability and, importantly, neither the T cell factor nor IgE can passively sensitize mast cell-deficient mice. The fact that neither IgE nor PCLF was active in two mutant strains of mice with completely different defects that each lead to mast cell deficiency makes it unlikely that another associated defect not pertaining to mast cells was responsible for the inability of either strain to serve as suitable recipients. This argues that the mechanism of action of PCLF, like that of IgE, is mast cell dependent. Moreover, morphologic evidence of mast cell activation occurs at sites of skin reactions that are mediated by PCLF (S. Kraeuter-Kops, H. Van Loveren, R. W. Rosenstein, W. Ptak, and P. W. Askenase, unpublished results).

Intravenous administration of IgE passively sensitized recipients for prolonged reactivity, whereas similarly administered PCLF was only able to passively sensitize

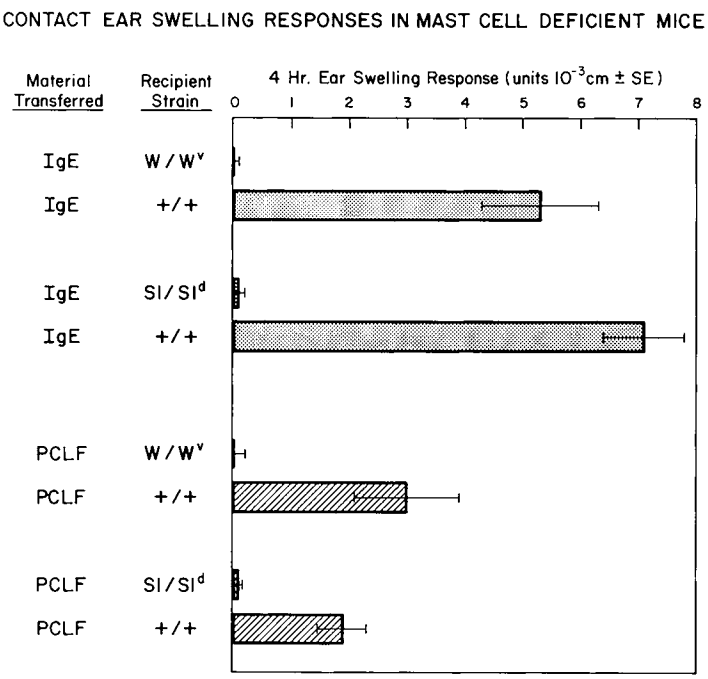

FIG. 7. Neither IgE nor PCLF transfers the ability to elicit ear swelling responses in mast cell deficient mice. Separate groups of mast cell deficient mice of two types (W/W^v and Sl/Sl^d), and their appropriate +/+ controls, were injected intravenously with 33 μg IgE, 75 μg PCLF, or nothing (12 mice of each type were used at 4 per group). Immediately thereafter mice were challenged on both ears with 0.8% PCL, and 4 h later ear swelling responses were measured with a micrometer. Ear swelling responses in noninjected mast cell deficient mice of both types and their appropriate +/+ controls were subtracted from the ear swelling response of the corresponding mice that were injected intravenoulsy.

recipients for a short time. Prolonged sensitization is expected with IgE antibody. This is a manifestation of the unique ability of its Fc portion to bind with high affinity to specialized Fc receptors on the surface of skin mast cells. The simplest explanation of our findings is that PCLF passively sensitizes mast cells for a short time. However, we have not yet been able to demonstrate that PCLF can passively sensitize mast cells directly, using either PCA reactions in vivo, or release of serotonin from murine mast cell suspensions in vitro. Since the IgE hybridoma antibody is very active in both assays, this suggests that the mechanism of mast cell activation by PCLF is different than the mechanism of mast cell activation by IgE.

The active moiety in PCLF has a molecular weight of 70,000 or less (7), is derived from lymphoid cells depleted of B cells (7), has no Ig heavy or light chain antigenic determinants (7), and has antigenic determinants found on T cells and on antigen-specific T cell factors (7, 14–16). Despite these findings indicating that PCLF is T cell derived and not an Ig, the striking resemblence between the biologic activity of PCLF and IgE necessitated rigorous experiments to rule out the possibility that the active moiety in PCLF was due to contamination with IgE or that PCLF was a fragment or a potential subclass of IgE (24). These studies showed that no ε heavy chain determinants were found in PCLF, using either a direct-binding ELISA (7) or a competitive binding radioimmunoassay (Fig. 4). Affinity chromatography using

specific anti-IgE and anti-PCLF antibodies linked to Sepharose showed complete separation of the ability of IgE and the active moiety in PCLF to passively sensitize for elicitation of immediate ear swelling responses. We ruled out the possibility that the particular anti-IgE column we used could fail to bind some IgE clonotypes, or that the anti-PCLF column could bind an IgE subclass. This was done by applying a hyperimmune polyclonal IgE antibody activity (as measured by PCA titrations) to the anti-IgE and anti-PCLF affinity columns. Thus PCLF, despite the fact that its biologic activity resembles that of IgE, is a T cell factor that is clearly distinct from IgE antibody.

The impetus for this work was the finding that T cell-dependent activation of mast cells is required for elicitation of delayed-type hypersensitivity responses (3–6). The finding of T cell-derived antigen-binding factors that resemble the in vivo activity of IgE antibody provides a mechanism by which T cell activation of mast cells may occur in cell-mediated immunity. This finding also raises the possibility that similar T cell-dependent mechanisms may underly immediate hypersensitivity-like responses in which appropriate IgE antibodies have not been found, such as some types of asthma, rhinitis, urticaria, or food allergy. T cell factors that can mediate immediate mast cell-dependent responses that feature increased vascular permeability could participate in some of these conditions.

Summary

T cell-dependent activation of resident tissue mast cells is required for the elicitation of delayed-type hypersensitivity skin reactions in mice. A T cell-derived antigen-binding factor that transfers the ability to elicit an immediate hypersensitivity-like skin reaction is described and compared with a hybridoma IgE antibody. Both the T cell factor and IgE mediate reactions with increased vascular permeability and both are mast cell dependent, as they are inactive in two different types of mast cell deficient mice (W/W^v and Sl/Sl^d). The T cell factor was distinguished from IgE by affinity chromatography using specific anti-IgE and anti-factor antibodies and by a shorter duration of passive sensitization. The T cell factor is a suitable candidate for participation in the mechanism by which T cells activate mast cells in delayed-type hypersensitivity.

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References

1. Huber, B., O. Devinsky, R. K. Gershon, and H. Cantor. 1976. Cell-mediated immunity. Delayed-type hypersensitivity and cytotoxic responses are mediated by different T cell subclasses. *J. Exp. Med.* **143:**1534.
2. Vadas, M. A., J. F. A. P. Miller, I. F. C. McKenzie, S. E. Chism, F. W. Shen, E. A. Boyse, J. R. Gamble, and A. W. Whiteland. 1976. Ly and Ia antigen phenotypes of T cells involved in delayed-type hypersensitivity and in suppression. *J. Exp. Med.* **144:**10.
3. Gershon, R. K., P. W. Askenase, and M. D. Gershon. 1975. Requirement for vasoactive amines in the production of skin reactions of delayed-type hypersensitivity. *J. Exp. Med.* **142:**732.

4. Schwartz, A., P. W. Askenase, and R. K. Gershon. 1977. The effect of locally injected vasoactive amines on the elicitation of delayed-type hypersensitivity. *J. Immunol.* **118:**159.
5. Askenase, P. W., S. Bursztajn, M. D. Gershon, and R. K. Gershon. 1980. T cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J. Exp. Med.* **152:**1358.
6. Askenase, P. W., C. M. Metzler, and R. K. Gershon. 1982. Localization of leukocytes in sites of delayed-type hypersensitivity and in lymph nodes: dependence on vasoactive amines. *Immunology.* **47:**239.
7. Ptak, W., P. W. Askenase, R. W. Rosenstein, and R. K. Gershon. 1982. Transfer of an antigen-specific immediate hypersensitivity-like reaction with an antigen binding factor produced by T cells. *Proc. Natl. Acad. Sci. USA.* **79:**1969.
8. Liu, F. T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenyl specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* **124:**2728.
9. Eshhar, Z., M. Ofarim, and T. Waks. 1980. Generation of hybridomas secreting murine reaginic antibodies of anti-DNP specificity. *J. Immunol.* **124:**775.
10. Bottcher, I., G. Hammerling, and J. F. Kapp. 1978. Continuous production of monoclonal mouse IgE antibodies with known allergeneic specificity by a hybrid cell line. *Nature (Lond.).* **275:**761.
11. Axen, R., J. Porath, and S. Ernback. 1967. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature (Lond.).* **214:**1302.
12. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:**248.
13. Asherson, G. L., and W. Ptak. 1968. Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. *Immunology.* **15:**405.
14. Cone, R. E., R. W. Rosenstein, J. H. Murray, G. M. Iverson, W. Ptak, and R. K. Gershon. 1981. Characterization of T cell surface proteins bound by heterologous antisera to antigen-specific T cell products. *Proc. Natl. Acad. Sci. USA.* **78:**6411.
15. Cone, R. E., R. W. Rosenstein, J. Tite, W. Ptak, and R. K. Gershon. 1983. Heterologous antisera to Lyt-1^+2^--derived antigen-binding factor detect a subfactor of an antigen-specific suppressor factor and cell surface proteins on Ly-1^+2^-, and Ly-1^-2^+ T cells. *J. Immunol.* In press.
16. Binz, H., and H. Wigzell. 1981. T cell receptors with allomajor histocompatability complex specificity from rat and mouse. Similarity of size, plasmin susceptibility, and localization of antigen-binding region. *J. Exp. Med.* **154:**1261.
17. Rosenstein, R. W., J. H. Murray, R. E. Cone, W. Ptak, G. M. Iverson, and R. K. Gershon. 1981. Isolation and partial characterization of an antigen-specific T cell factor associated with the suppression of delayed-type hypersensitivity. *Proc. Natl. Acad. Sci. USA.* **78:**5821.
18. Askenase, P. W., and E. J. Leonard. 1970. Solid phase radioimmunoassay of human beta-1-c globulin. *Immunochemistry.* **7:**29.
19. Cone, R. E., and W. Brown. 1976. Isolation of membrane associated immunoglobulins from T lymphocytes by non-ionic detergents. *Immunochemistry* **13:**571.
20. Kitamura, Y., S. Go, and K. Hatanaka. 1978. Decrease of mast cells in W/W^v mice and their increase by bone marrow transplantation. *Blood.* **52:**447.
21. Kitamura, Y., and S. Go. 1979. Decreased production of mast cells in Sl/Sl^d anemic mice. *Blood.* **53:**492.
22. Kitamura, Y., K. Hatanaka, M. Shimada, S. Go, and H. Matsuda. 1979. Local control of mast cell differentiation in mice. *In* Experimental Hematology Today. S. J. Baum and G. D. Ledney, editors. Springer-Verlog, New York. 45.
23. Kitamura, Y., M. Shimada, S. Go, H. Matsuda, K. Hatanaka, and M. Seki. 1979.

Distribution of mast cell precursors in hematopeoietic and lymphopoietic tissues of mice. *J. Exp. Med.* **150:**482.

24. Lehrer, S. B., M. L. McCants, P. N. Farris, and H. Bazin. 1981. Passive cutaneous anaphylaxis inhibition: evidence for heterogeneity in IgE mast cell interaction. *Immunology.* **44:**711.