The Journal of Immunology
RESEARCH ARTICLE | JULY 01 1980

Regulatory role of IgE-binding factors from rat T lymphocytes. I. Mechanism of enhancement of IgE response by IgE-potentiating factor. FREE

M Suemura; ... et. al

J Immunol (1980) 125 (1): 148–154.
https://doi.org/10.4049/jimmunol.125.1.148

0022-1767/80/1251-0148$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 125, No. 1, July 1980
Printed in U.S.A.

REGULATORY ROLE OF IgE-BINDING FACTORS FROM RAT T LYMPHOCYTES

I. Mechanism of Enhancement of IgE Response by IgE-Potentiating Factor[1]

MASAKI SUEMURA, JUNJI YODOI, MITSUOMI HIRASHIMA, AND KIMISHIGE ISHIZAKA

From the Department of Medicine and Microbiology, The Johns Hopkins University, School of Medicine at the Good Samaritan Hospital, Baltimore, Maryland 21239

T lymphocytes in the mesenteric lymph nodes of rats infected with *Nippostrongylus brasiliensis* spontaneously released a soluble factor that selectively potentiated the IgE-forming cell response of antigen-primed cells to homologous antigen. The factor could enhance the IgE response of DNP-OA-primed cells to DNP-HSA and T cell-replacing factor. In contrast, the treatment of OA-primed T cells with the factor failed to enhance either the IgE or IgG response of the mixture of DNP-KLH primed cells and OA-primed T cells to DNP-OA. The results collectively suggested that the target cells of the IgE-potentiating factor are B cells. Indeed, IgE potentiating factor was absorbed by B cells rather than T cells or thymocytes. Evidence was obtained that IgE-potentiating factor could be absorbed by IgE-bearing B cells or IgE-coupled Sepharose, indicating that the factor had affinity for IgE. It appeared that the potentiating factor bound to IgE-bearing B cells and selectively enhanced the differentiation of IgE-B cells to IgE-forming cells. It was also found that the major source of the factor was $Fc_\epsilon R$-bearing T cells.

It has been established that infection of rats with *Nippostrongylus brasiliensis* (Nb)[2] causes nonspecific potentiation of the IgE antibody response to unrelated antigens such as ovalbumin (OA) or keyhole limpet hemocyanin (KLH) (1-3). Bloch *et al.* (3) have shown that the potentiation of anti-KLH antibody formation after Nb-infection was restricted to IgE antibodies; neither the IgG1 nor IgG2 antibody response was altered after the infection. Subsequently, Jarrett and Ferguson (4) suggested that T cells were essential for the potentiation of IgE response in the infected rats. Indeed, our previous studies have shown that T cells from Nb-infected rats could potentiate the *in vitro* IgE-forming cell response of DNP-OA-primed cells to homologous conjugate without affecting the IgG-forming cell response (5). It was also found that mesenteric lymph node (MLN) cells from Nb-infected rats released a soluble factor that selectively enhanced the IgE response of DNP-OA primed cells to homologous antigen. The IgE-potentiating factor was detected in 24-hr culture supernatants of the MLN cells. Evidence was obtained that the IgE-potentiating factor was derived from T cells rather than B cells, and that the factor was different from T cell-replacing factor (TRF), which was obtained by stimulation of antigen-primed T cells with antigen (5).

The present experiments were undertaken to elucidate the mechanisms of potentiation by the IgE-potentiating factor and to identify the source of this factor. The results show that the factor is derived from Fc_ϵ-receptor ($Fc_\epsilon R$)-bearing T cells and suggest that the binding of this factor to IgE-bearing B cells facilitates the differentiation of IgE-B memory cells.

MATERIALS AND METHODS

Antigens. Crystalline ovalbumin (OA) and crystalline human serum albumin (HSA) were purchased from Nutritional Biochem. Corp., Cleveland, Ohio. Crystalline bovine serum albumin (BSA) was obtained from Armour Pharmaceutical Co., Chicago, Ill. Keyhole limpet hemocyanin was purchased from Pacific Bio-Marine Supply Co., Venice, Calif., and was purified by the method of Campbell *et al.* (6). 2,4-dinitrobenzene sulfonic acid was obtained from Eastman Organic Chemicals, Rochester, N. Y. Dinitrophenyl (DNP) derivatives of OA, HSA, and KLH were prepared by the method of Eisen (7). DNP-OA and DNP-HSA contained 6.2 and 16 DNP groups per molecule, respectively. DNP-KLH contained 7 DNP groups per 100,000 m.w. unit of KLH. Nippostrongylus antigen was prepared from adult Nb worms by the method previously described (8). The antigen employed in the present experiments were the same material as that described in a previous article (5).

Immunoglobulin and anti-immunoglobulin. Rat monoclonal IgE (IR-162) and the γ-globulin fraction of a monospecific rabbit anti-IgE serum (anti-IR 183) were the same preparations as those described in a previous article (9). The IgG fraction of a rabbit antiserum specific for rat IgG2, a preparation of specifically purified goat anti-rat Ig antibodies (polyvalent anti-rat Ig) and fluoresceinated $F(ab')_2$ fragments of the anti-Ig antibod-

Received for publication January 14, 1980.
Accepted for publication March 26, 1980.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by Research Grants AI-14784 and AI-11202 from the United States Public Health Service. This is publication No. 388 from the O'Neill Laboratories at the Good Samaritan Hospital.
[2] Abbreviations used in this paper: OA, ovalbumin; HSA, human serum albumin; MLN, mesenteric lymph nodes; Nb, *Nippostrongylus brasiliensis*; KLH, keyhole limpet hemocyanin; RGG, rabbit IgG; TRF, T cell-replacing factor; $Fc_\epsilon R$, Fc receptors for IgE; CFA, complete Freund's adjuvant; BSS, balanced salt solution; CFS, cellfree culture supernatant; E'-IgE, ox erythrocytes coated with rat IgE; RFC, rosette-forming cells.

ies, were the same preparations as those employed in previous experiments (5, 10). Specifically purified goat antibodies against rabbit IgG (anti-RGG) were previously described (11). A fluoresceinated γ-globulin fraction of a goat anti-rabbit IgG was purchased from Meloy Laboratories Inc., Springfield, Va. A rabbit antiserum specific for rat T cells (12) was kindly supplied by Dr. K. Kikuchi, Sappolo Medical School, Sappolo, Japan. The specificity of the antiserum was confirmed in a previous article (11).

Immunosorbents. Rat IgE was coupled to CL Sepharose 4B (Pharmacia, Uppsala, Sweden) by the method described by Conrad and Froese (13); 15 mg of pure rat IgE in phosphate-buffered saline (PBS), pH 7.4, were added to 2 ml of CL Sepharose, which had been activated by cyanogen bromide. Control Sepharose beads were coupled to 25 mg of BSA.

Infection and immunization. Inbred Lewis strain rats were purchased from Microbiological Associates, Walkersville, Md. They were infected with 2400 to 2600 Nb larvae via a subcutaneous injection as described by Ogilvie (14). Mesenteric lymph node (MLN) cells were obtained 2 or 4 weeks after infection.

Groups of Lewis strain rats were immunized twice with 5 μg of DNP OA included in complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich.) or 10 μg DNP-KLH in CFA at a 4-week interval. MLN cells were obtained 2 to 3 weeks after the last immunization. In order to obtain OA-primed T cells, a group of rats was immunized with 5 μg OA included in CFA, and their MLN cells were obtained 3 weeks after immunization.

Cell fractionation. Fractionation of MLN cells into T and B cell fractions was carried out by a modification of the method of Schlossman and Hudson (15). Detailed procedures were described in a previous article (11). The T cell-enriched fraction was depleted of residual Ig-bearing cells by fractionation on tissue culture dishes coated with anti-rat Ig (11), which were prepared by the method of Mage *et al.* (16).

In some experiments, the T cell fraction of MLN cells were obtained by the method of Julius *et al.* (17) by using nylon wool columns (LP-1 Leukopack leukocyte filter, Fenwal Laboratories, Morton Grove, Ill.). Detailed procedures for the fractionation of rat lymphocytes were described in a previous article (18). The T cell-enriched fraction was further purified by using anti-Ig-coated dishes. In all experiments described in this paper, the proportion of Ig-bearing cells in the final T cell fractions and B cell fractions were 0.1 to 0.5% and 75 to 90%, respectively. About 90% of the total cells in the T cell fraction were stained by immunofluorescence by using anti-T cell antibodies.

Depletion of IgE-bearing cells was carried out by the method described in a previous article (11). Briefly, MLN cells were treated with anti-rat IgE. The cells were suspended in Hanks' balanced salt solution (BSS) containing 10 mM sodium azide and 5% fetal calf serum, and the suspension was placed in tissue culture dishes coated with specifically purified anti-rabbit IgG (RGG). A portion of the anti-IgE-treated cells were placed in culture dishes coated with normal goat IgG. After 1 hr incubation at 4°C, nonadherent cells were recovered from each dish.

Lymphocytes bearing $Fc_\epsilon R$ were depleted by specific adsorption of the cells to IgE-coated dishes that were prepared by successive treatment with 30 μg/ml rat IgE, 10 μg/ml of the $F(ab')_2$ fragments of rabbit anti-rat IgE, and 30 μg/ml rat IgE. Procedures for the fractionation were described in a previous article (19).

Cell cultures. An *in vitro* culture system for the development of Ig-forming cells was exactly the same as that described in a previous article (11). Thus, MLN cells of DNP-OA-primed rats were cultured in Click's medium (20), supplemented with 10% normal rat serum, 5×10^{-5} M 2-mercaptoethanol, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Cultures were set up in flat-bottomed Micro-Test II culture plates (Falcon Plastics, Oxnard, Calif.). Ten replicate microplate wells were used for each culture condition, and each well contained 10^5 viable nucleated cells in 0.2 ml in the presence of 1 μg/ml DNP-OA or DNP-HSA. The culture plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Based on the results of previous experiments (11), Ig-forming cells were enumerated after 5 days, which was the peak response.

In order to assess the effect of IgE-potentiating factor on the IgE response, a suspension of DNP-OA-primed cells was mixed with an equal volume of a 1:4 dilution (except for the experiment in Fig. 7) of cellfree culture supernatant (CFS) of MLN cells from Nb-infected rats (see below), and the cell suspension was cultured with DNP-OA for 5 days.

Preparation of culture supernatant. MLN cells obtained 2 weeks after infection with Nb were employed as a source of IgE-potentiating factor. Either MLN cells or their T cell fraction were suspended in Click's medium supplemented with 2.5% normal rat serum, and a cell suspension containing either 5×10^7 cells/ml or 2×10^7 cells/ml were cultured for 24 hr. CFS recovered by centrifugation for 30 min at 10,000 rpm were dialyzed against Click's medium, and stored at −40°C until use. In some experiments, CFS preparations were filtered through a Diaflo 50A membrane with an Amicon centriflo system, and were stored at −40°C.

T cell-replacing factor (TRF) was obtained from MLN cells of rats immunized with 15 μg Nb antigen included in CFA. MLN cells were obtained 4 weeks after immunization and a cell suspension (5×10^7 cells/ml) was incubated with 10 μg/ml of Nb antigen. After 24 hr, the culture supernatant was recovered and stored at −40°C until use.

Immunofluorescence: A) *cell surface staining.* The proportions of Ig-bearing cells and IgE-bearing cells were determined by either direct or indirect immunofluorescence with fluoresceinated $F(ab')_2$ fragments of anti-rat Ig or rabbit antibodies specific for rat IgE (11). In each cell suspension examined for immunofluorescence, 1000 to 2000 cells were viewed in both tungsten and UV light with a Leitz Ortholux microscope (E. Leitz Inc., Rockleigh, N. J.), equipped with an Osram HG 200 mercury lamp, BG 38 and KP 490 excitation filters, and a K 530 barrier filter. The experimental error in the determination of the proportion of stained cells was ± 10%.

B) *Enumeration of Ig-forming cells.* One to 3×10^5 viable cells were layered on a glass slide by using a Shandon cytocentrifuge and fixed. The smears were treated with anti-IgE or anti-IgG2, followed by fluoresceinated anti-RGG. Details for the staining procedures were described in a previous article (11). Only intensely stained plasma cells and blast cells were counted in duplicate slides. The experimental error in the determination of the proportion of stained cells was within ± 15% in all the experiments reported. Unless otherwise specified, the Ig-forming cell responses were expressed as the number of Ig-containing cells developed from 1×10^6 cells placed in the culture.

Rosette assay. Lymphocytes bearing $Fc_\epsilon R$ were enumerated by a rosette technique by using ox erythrocytes (Colorado Serum Co., Denver, Colo.) coated with rat monoclonal IgE (E'-IgE). Red cells coated with HSA were used to measure the level of nonspecific rosettes. The sensitized erythrocytes were prepared by the method of Gonzalez-Molina and Spiegelberg (21) with slight modifications. The procedures for rosette assays

were exactly the same as those described in a previous article (9). In order to test the ability of CFS to inhibit rosette formation, 30 μl of an appropriate dilution of CFS were added to 15 μl of 2% E'-IgE. After 30 min at 4°C, 15 μl of a lymphocyte suspension (10^7 cells/ml) were added to the mixture. The lymphocytes were obtained from a Nb-infected rat 2 to 3 weeks after infection. After incubation of lymphocytes with indicator cells for 10 min at 37°C, the cell suspensions were centrifuged at 90 × G for 5 min and kept at 0°C for 90 min. Pellets were gently mixed with 0.1% toluidine blue in PBS and an aliquot was examined in a hemocytometer. Usually, 600 to 1000 cells were counted to determine the percentage of rosette forming cells (RFC). The number of IgE-RFC was obtained by subtracting the number of nonspecific RFC with E'-HSA from the number of E'-IgE-RFC. The experimental error in the determination of the proportion of RFC was less than ± 15%.

RESULTS

Experiments were carried out to determine whether the IgE-potentiating factor might affect either an early stage or late stage of B cell differentiation. Aliquots of DNP-OA-primed cells were cultured with DNP-OA for 5 days, and a 24-hr CFS of MLN cells from Nb-infected rats was added to the culture at different intervals after initiation of the culture. The final concentration of CFS in the culture was 1:8. As shown in Figure 1, the IgE-forming cell response was markedly enhanced when CFS was added at the beginning of the culture. The potentiation of the IgE response was much less if CFS was added 24 hr after the initiation of the culture. The same CFS added at day 2 or later failed to enhance the IgE response. The magnitude of the IgG response was not affected by the addition of CFS.

In view of these findings, we determined whether exposure of DNP-OA-primed cells to CFS for only the first 24 hr of culture was sufficient to enhance the IgE response. The experimental protocol and results are shown in Figure 2. Thus, aliquots of DNP-OA-primed cells were incubated for 24 hr with DNP-OA in the presence or absence of CFS. Cells were washed three times with Hanks' BSS, resuspended in culture medium, and cultured for 4 days with DNP-OA. It can be seen that incubation of MLN cells with CFS during the first 24 hr was sufficient to enhance the IgE response 2-fold, although the presence of CFS for the entire period was more effective.

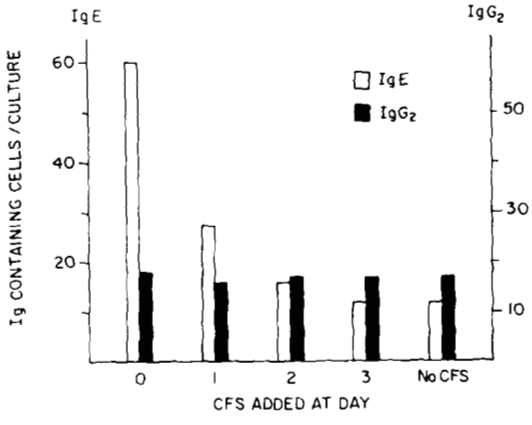

Figure 1. Potentiation of IgE response of DNP-OA-primed cells by CFS added at an early stage of culture. DNP-OA-primed cells were cultured with DNP-OA for 5 days. The numbers of Ig-containing cells represent those developed in 1 × 10^6 cells placed in the culture. Selective enhancement of IgE-forming cell response was not obtained if CFS was added at day 2 or later.

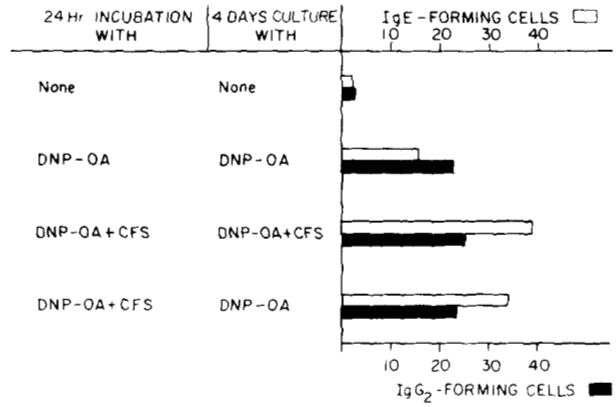

Figure 2. Potentiation of IgE response by exposure of DNP-OA-primed cells to CFS for 24 hr. DNP-OA-primed MLN cells were incubated for 24 hr with an equal volume of a 1:4 dilution of CFS in the presence of DNP-OA. The control culture (2nd row) contained no CFS. After washing, cells were cultured with DNP-OA for 4 days in the presence (third row) or absence (fourth row) of CFS. Both IgE-forming cells and IgG2-forming cells were enumerated.

Target cells for IgE potentiating factor. In order to determine whether CFS could potentiate the number of IgE-forming cells even when carrier-primed T cells were not present, its effect was studied on cultures of DNP-OA primed cells with DNP-HSA and TRF, since such cultures do generate an Ig-forming cell response (5). The protocol of these experiments, and representative results, are shown in Figure 3. Thus, aliquots of MLN cells from DNP-OA-primed rats were incubated for 24 hr with DNP-HSA in the presence or absence of CFS, which contained IgE potentiating factor. After washing, the cells were resuspended in fresh medium containing DNP-HSA and TRF, and the cells were cultured for 4 days. The concentration of TRF in the culture was chosen, based on preliminary experiments, such that the magnitude of IgE-response was suboptimal. Indeed, neither an IgE nor an IgG response was obtained unless TRF was added to the culture (*cf.* groups A *vs* B). It is apparent in Figure 3 that IgE-potentiating factor significantly enhanced the IgE response by DNP-heterologous carrier and TRF (groups C *vs* B). The experiments also showed that incubation of DNP-OA-primed cells with CFS alone during the first 24 hr incubation significantly enhanced the IgE response (groups D *vs* E).

Similar experiments were carried out with the T cell fraction of MLN cells as a source of IgE-potentiating factor. MLN cells of Nb-infected rats were fractionated to obtain a T cell-enriched fraction and the cells were cultured for 24 hr to obtain CFS. It is apparent in Figure 4, that the CFS preparation could potentiate the IgE response of DNP-OA-primed cells to DNP-HSA and TRF, without affecting the IgG response. The results indicated that IgE-potentiating factor and TRF collaborated for the optimal IgE response, and suggested that the potentiating factor might affect B cells.

Experiments were carried out to exclude the possibility that the IgE-potentiating factor might stimulate carrier-specific helper cells. The T cell fraction of MLN cells from OA-primed rats were incubated for 24 hr with the same CFS as that employed in the experiment shown in Figure 3. After washing, either the CFS-treated or untreated OA-primed T cells (1 × 10^5) were mixed with an equal number of DNP-KLH-primed MLN cells and the mixtures were cultured for 5 days in the presence of DNP-OA. As a control, 2 × 10^5 DNP-KLH-primed cells were cultured with DNP-OA. The mixture of DNP-KLH-primed cells and OA-primed T cells responded to DNP-OA

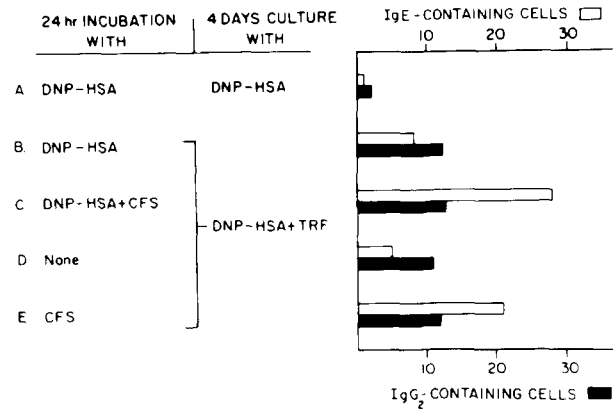

Figure 3. IgE-potentiating factor enhances IgE response of DNP-OA-primed cells to DNP-HSA and T cell replacing factor (TRF). DNP-OA-primed cells were incubated with DNP-HSA alone (B), DNP-HSA + CFS (C), or CFS alone (E) for 24 hr. Cells were washed and cultured for 4 days in the presence of DNP-HSA and TRF. A 1:20 dilution of the supernatant of an antigen-stimulated culture of Nb-antigen-primed MLN cells was used as the source of TRF.

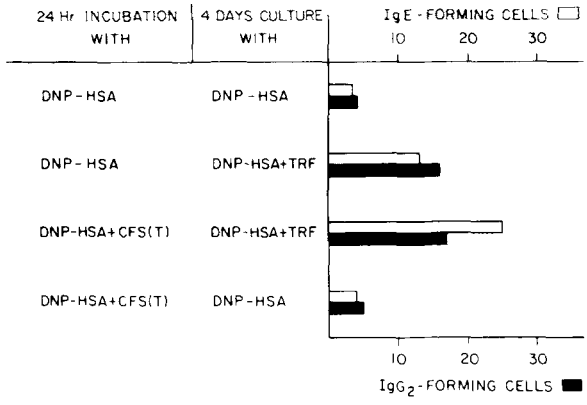

Figure 4. Selective potentiation of IgE response by CFS from T cells. CFS was obtained from the T cell fraction of MLN cells from Nb-infected rats. DNP-OA-primed cells were incubated with DNP-HSA in the presence or absence of CFS. Cells were washed and cultured for 4 days with DNP-HSA in the presence or absence of TRF. CFS from T cells selectively enhanced the IgE response (third row) but failed to replace carrier-primed cells (fourth row).

with an IgE and IgG-forming cell response, whereas DNP-KLH primed cells alone plus DNP-OA failed to do so. The treatment of OA-primed T cells with CFS failed to enhance either the IgE- or IgG-forming cell responses. The number of IgE-containing cells developed in the cultures with CFS-treated OA-primed T cells was 24 per 10^6 cells seeded, and was comparable to those developed in the cultures with untreated OA-primed T cells (23 per 10^6 cells seeded). The results suggested that the target cells of IgE-potentiating factor were B cells rather than T helper cells.

Absorption of IgE-potentiating factor. In order to prove that IgE-potentiating factor has affinity for a subset of B cells, attempts were made to absorb the factor with lymphocytes from various sources. Preliminary experiments showed that MLN cells from Nb-infected animals failed to release IgE-potentiating factor at 4°C. Thus, MLN cells were obtained from rats 4 weeks after Nb infection, and fractionated into T cell-enriched and B cell-enriched fractions. One-milliliter aliquots of a 1:4 dilution of CFS were absorbed with 10^8 cells of each fraction at 4°C, and the supernatant was assessed for its ability to potentiate the IgE response of DNP-OA-primed cells to DNP-OA. The results shown in Figure 5 indicate that IgE-potentiating factor is absorbed by the B cell fraction but not by T cells. We have also tested the ability of thymocytes and normal mesenteric lymph node cells to absorb IgE-potentiating factor. In the experiments shown in Figure 6, a 1:4 dilution of CFS was absorbed at 4°C with 4×10^8 cells from various sources, and the supernatants were assessed for their ability to potentiate IgE responses. Repeated experiments of the same design indicated that MLN cells from Nb-infected rats were more effective than normal MLN cells in absorption of IgE-binding factor.

Since our previous experiments have shown that the proportion of IgE-bearing cells in the MLN cells markedly increased after Nb-infection (22), we anticipated the possibility that IgE-bearing B cells might be responsible for the absorption of IgE-potentiating factor. In order to examine this possibility, we have tested the ability of IgE-B cell-depleted fractions to absorb IgE-

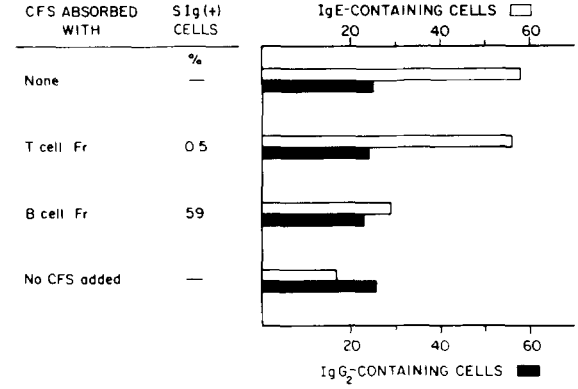

Figure 5. Absorption of IgE-potentiating factor by B cells but not by T cells. T and B cell fractions were obtained from MLN cells of Nb-infected rats. One-milliliter aliquots of CFS (1:4 dilution) were absorbed with 10^8 nucleated cells at 4°C for 2 hr. Supernatants were assessed for their ability to potentiate the IgE response of DNP-OA-primed cells to DNP-OA. IgE-potentiating factor was absorbed by the B cell-enriched fraction but not by T cells.

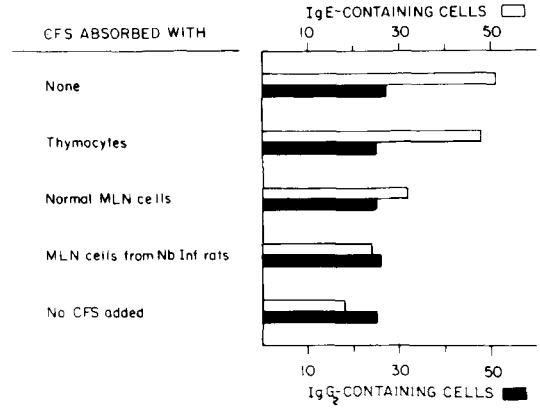

Figure 6. Absorption of IgE-potentiating factor by MLN cells but not by thymocytes. One-milliliter aliquots of CFS (1:4 dilution) were absorbed with 4×10^8 nucleated cells. Cell suspensions were kept at 4°C for 2 hr and supernatants were assessed for their ability to potentiate the IgE response, by the same procedure as described in Figure 5. The proportion of B cells in MLN cells from normal and Nb-infected rats were comparable (20 to 21% of total cells). The proportion of IgE-B cells in the latter (infected) preparation was 9.5% as compared with 4.2% for normals.

potentiating factor. Thus, MLN cells from Nb-infected rats were treated with anti-IgE, and an aliquot of antibody-coated cells was depleted by anti-RGG-coated dishes. A CFS preparation was absorbed with either untreated MLN cells, anti-IgE-treated cells, or an IgE-B cell-depleted cell fraction. Since the proportion of B cells in the IgE-B cell-depleted fraction was about two-thirds of that in the original cell suspension, CFS was absorbed with 3×10^8 cells of this fraction or 2×10^8 cells of the original cell suspension. After 2 hr at 4°C, supernatants were recovered and assessed for the ability to potentiate the IgE response. It can be seen in Table I that the IgE-B cell-depleted fraction was less effective than anti-IgE-treated cells in absorption of IgE-potentiating factor. The results supported the idea that IgE-bearing B cells were responsible for the absorption.

In order to test the possibility that the IgE-potentiating factor may have affinity for IgE, attempts were made to absorb IgE-potentiating factor with IgE-coated Sepharose. Since the m.w. of the IgE-potentiating factor is between 10,000 to 25,000 (5), CFS was passed through an Amicon Diaflo 50A membrane, and the filtrate was concentrated 5-fold by ultrafiltration. One-milliliter aliquots of the preparation were mixed with 0.25 ml of either IgE-coated Sepharose or BSA-coated Sepharose. The mixtures were rotated at room temperature for 90 min and then centrifuged to recover supernatants. The ability of the absorbed and unabsorbed preparations to potentiate the IgE response was assessed by using DNP-OA-primed cells. A 1:10 dilution of each preparation was added to DNP-OA-primed cells and the cells were cultured for 5 days in the presence of DNP-OA. As shown in Figure 7, IgE-potentiating factor was absorbed by IgE-Sepharose but not by BSA-Sepharose, indicating that the soluble factor had affinity for IgE.

Source of IgE-potentiating factor. Previous experiments from our laboratory have shown that a subpopulation of T cells in MLN from Nb-infected rats bear $Fc_\epsilon R$ (9), and that the $Fc_\epsilon R(+)$ T cells release a soluble factor that inhibits rosette formation of $Fc_\epsilon R(+)$ cells with E′-IgE (23). The rosette-inhibiting factor could be absorbed with IgE-coupled Sepharose, indicating that the factor has affinity for IgE. Since the IgE-potentiating factor was also absorbed by IgE-Sepharose, we anticipated that the factor might be derived from $Fc_\epsilon R(+)$ T cells. Thus, MLN cells from Nb-infected rats were passed through a glass wool column, and nonadherent cells were placed in IgE-coated dishes to deplete $Fc_\epsilon R(+)$ cells. The proportion of $Fc_\epsilon R(+)$ cells in the lymphocyte fraction was 19.3%, whereas

TABLE I

Effect of IgE-B cell depletion on the absorption of IgE-potentiating factor

CFS Absorbed with[a]	Cells for Absorption		Ig-Forming Cells[b]	
	B cells	IgE-B cells	IgE	IgG_2
	%	%		
None			30	18
MLN cells (2×10^8)	19.8	9.6	14	17
Anti-IgE treated MLN cells (2×10^8)	19.8	9.6	16	18
IgE-B cell depleted Fr. (3×10^8)	12.3	3.5	25	18
No CFS			12	18

[a] One milliliter of a 1:4 dilution of CFS was absorbed with 2×10^8 MLN cells or 3×10^8 IgE-B cell-depleted fraction at 4°C.

[b] An equal volume of supernatant was added to a suspension of DNP-OA-primed cells and mixtures were cultured for 5 days with DNP-OA. The values represent the number of Ig-forming cells developed from 1×10^6 cells placed in the culture.

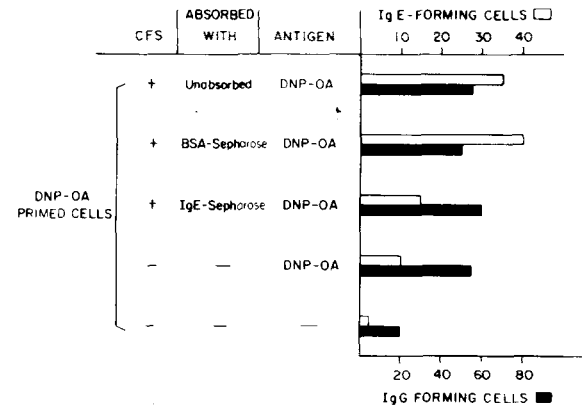

Figure 7. Absorption of IgE-potentiating factor by IgE-coupled Sepharose. CFS were filtered through a Diaflo 50A membrane and concentrated five times by ultrafiltration. One-milliliter aliquots of the preparation were absorbed with 0.4 ml of either BSA-Sepharose or IgE-Sepharose and supernatants (at a 1:10 dilution) were assessed for their ability to potentiate the IgE response of DNP-OA-primed cells to DNP-OA.

the proportion diminished to 5.7% in the $Fc_\epsilon R$-depleted fraction. Both the unfractionated lymphocytes and the $Fc_\epsilon R$-depleted fraction were suspended in culture medium (2×10^7 nucleated cells/ml) and incubated at 37°C for 24 hr to obtain culture supernatants. Each CFS preparation was passed through a Diaflo 50A membrane, and the filtrate was assessed for its ability to potentiate the IgE response of DNP-OA-primed cells. It can be seen in Figure 8 that the culture supernatants of the $Fc_\epsilon R$-depleted fraction potentiated the IgE response much less than CFS of unfractionated lymphocytes. The same filtrates were assessed for their ability to inhibit rosette formation of $Fc_\epsilon R(+)$ cells with E′-IgE. In the inhibition assay, lymphocytes from another Nb-infected rat were used as a source of $Fc_\epsilon R(+)$ cells. As shown in Table II, the amount of rosette-inhibiting factor produced by the $Fc_\epsilon R$-depleted fraction was less than one-third of that formed by unfractionated lymphocytes.

DISCUSSION

Previous studies have shown that the *in vitro* IgE-forming cell response of DNP-OA primed lymphocytes to homologous antigen was markedly enhanced by the addition to the culture of T cells from Nb-infected rats (5). It was also found that the potentiating effect of T cells was mediated by soluble factor(s). The factor was spontaneously released into culture fluids and selectively potentiated the IgE response of DNP-OA-primed cells to homologous antigen. The IgE-potentiating factor was different from the TRF that was obtained by stimulation of carrier-primed T cells with specific carrier. The CFS from unstimulated cultures failed to enhance the Ig response of DNP-OA-primed cells to DNP-HSA, whereas TRF did enhance. Furthermore, the m.w. of IgE-potentiating factor was significantly smaller than that of TRF (5).

In the present experiments, we studied the mechanism through which the IgE-potentiating factor enhanced the IgE response. The effect of IgE-potentiating factor on the IgE response was reproduced in an *in vitro* system in which TRF was employed in place of carrier-specific T cells. Thus, CFS of MLN cells from Nb-infected rats, or those obtained from their T cell fraction, enhanced the IgE response of DNP-OA primed cells to DNP-heterologous carrier and TRF (Fig. 3, 4). As expected, 24-hr pretreatment of DNP-KLH-primed cells with CFS selectively enhanced the IgE response of the mixtures of

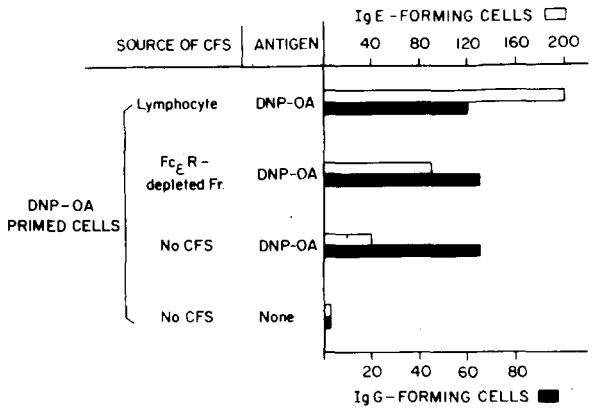

Figure 8. Source of IgE-potentiating factor. MLN lymphocytes from Nb-infected rats were depleted of $Fc_\epsilon R(+)$ cells (c.f. Table II). Lymphocytes and their $Fc_\epsilon R(+)$-depleted fractions were cultured for 24 hr to obtain CFS. CFS were filtered through Diaflo 50A membranes and assessed for their ability to potentiate the IgE response of DNP-OA-primed cells to DNP-OA. CFS from the $Fc_\epsilon R$-depleted fractions had much less ability to potentiate the IgE response.

TABLE II

Effect of $Fc_\epsilon R(+)$ cell-depletion on the spontaneous formation of IgE-binding factor by lymphocytes from Nb-infected rats[a]

Fraction	$Fc_\epsilon R(+)$	Rosette Inhibition by CFS[b]		
		Dilution	IgE-RFC	Inhibition
	%		%	%
Lymphocytes	19.3	1:2	2.8	79
		1:6	7.5	45
$Fc_\epsilon R$-depleted	5.7	1:2	9.6	29
		1:6	11.3	17
Medium control			13.6	

[a] Lymphocytes were obtained from mesenteric lymph nodes 2 weeks after Nb-infection.

[b] Cells were incubated for 24 hr in IgE-free medium to obtain culture supernatants, that were then filtered through Diaflo 50A membrane.

DNP-KLH primed cells and OA-primed cells to DNP-OA (data not shown). In contrast, pretreatment of OA-primed T cells with CFS failed to enhance the IgE response of the mixtures of OA-primed T cells and DNP-KLH-primed cells to DNP-OA. These findings suggested that IgE potentiating factor may stimulate B memory cells rather than T helper cells. This idea was supported by the fact that IgE-potentiating factor was absorbed by B cells rather than T cells (Fig. 5).

It was shown in a previous article (11) that B memory cells committed for IgE bear both IgE and IgM determinants on their surface, whereas B memory cells for IgG or IgM lack surface IgE. Enhancement of the IgE response by IgE-potentiating factor suggested that the factor may selectively stimulate IgE-bearing B cells. Indeed, MLN cells from infected rats, which contained a high proportion of IgE-bearing B cells, absorbed more IgE-potentiating factor than normal MLN cells. Furthermore, depletion of IgE-B cells from MLN cells from infected animals resulted in a marked decrease in the ability to absorb IgE-potentiating factor. These findings suggested that the factor had affinity for IgE-B cells. The present experiments also showed that IgE-potentiating factor could be absorbed by IgE-coupled Sepharose but not by BSA-coupled Sepharose. Although the results are not shown, recent experiments indicated that IgG-coated Sepharose failed to absorb IgE-potentiating factor. In our more recent experiments, which will be published separately, IgE-potentiating factor was absorbed to IgE-coupled Sepharose and was recovered from the beads at acid pH. It appears that the factor has affinity for IgE and binds to IgE-B cells through surface IgE. It is reasonable to speculate that selective potentiation of the IgE response by IgE-potentiating factor is due to the binding of the factor to IgE-B cells but not to B memory cells for the other isotypes.

Recent experiments from our laboratory have shown that T lymphocytes in the MLN from Nb-infected rats release a soluble factor that can inhibit rosette formation of $Fc_\epsilon R(+)$ lymphocytes with IgE-coated red cells. The factor was specifically absorbed with IgE-coated Sepharose, indicating that the factor had affinity for IgE (23). These observations together with the present findings suggest strongly that IgE-potentiating factor may be the IgE-binding factor detected by rosette inhibition. It was found that both the IgE-potentiating factor and IgE-binding factor have affinity for IgE, and have a m.w. of between 10,000 to 20,000 (5, 23). Furthermore, the major source of both factors appears to be $Fc_\epsilon R(+)$ T cells. Depletion of 70% of $Fc_\epsilon R(+)$ cells in the lymphocyte cell suspension before culture resulted in a two-thirds decrease in the amount of IgE-binding factor in the culture supernatant (Table II) and a concomitant marked decrease in the formation of IgE-potentiating factor (Fig. 8). It was shown in a previous article (9) that $Fc_\epsilon R(+)$ cells in MLN markedly increased after infection of rats with Nb, and that a significant proportion of $Fc_\epsilon R(+)$ cells in the infected animals are T lymphocytes. Failure of normal MLN cells to release IgE-potentiating factor is in agreement with the fact that $Fc_\epsilon R(+)$ T cells were undetectable in normal MLN. Spontaneous release of IgE-potentiating factor from $Fc_\epsilon R(+)$ T cells suggests a possible relationship between $Fc_\epsilon R$ on T cells and the soluble factor. One might speculate that the factor is a shed product of, or a fragment of, $Fc_\epsilon R$ on T cells.

It is not yet clear whether the IgE-binding factor is identical to IgE-potentiating factor. In our previous publication (23), it was shown that rat lymphocytes obtained 8 days after Nb-infection did not contain $Fc_\epsilon R(+)$ T cells and failed to release IgE-binding factor, but incubation of these cells with rat IgE resulted in the formation of the factor. However, more recent experiments, which will be published separately, indicated that the IgE-binding factor obtained by this procedure failed to potentiate, but instead suppressed the IgE response of DNP-OA-primed cells to DNP-OA. It appears that IgE-binding factors are heterogenous and that the biologic properties of the factors may be different depending on their source.

The present findings on IgE potentiating factor indicate that an optimal IgE response requires two subsets of T cells; one is a carrier-specific T cell that is common to all isotypes, and another subset is a $Fc_\epsilon R(+)$ T cell forming IgE-potentiating factor. Many investigators have suggested that two subsets of T helper cells participate in the antibody response. Marrack and Kappler (24, 25) as well as Tada *et al.* (26) provided evidence that there are two distinct functionally specialized sets of helper cells that may act synergically in most antibody responses. It was also reported that both carrier-specific helper T cells, and either allotype-specific helper or idiotype-specific helper cells, are involved in an antibody response (27, 28), and that two separate signals from the two types of T helper cells are required for an optimal antibody response of a certain allotype (idiotype). Janeway (29) generalized these concepts and predicted that one of the two subsets of helper T cells (Th1) is carrier-specific and acts through a hapten-carrier bridge with B cells, whereas the other subset of helper cells (Th2) has specificity both for Ig-determinants and for antigen,

but does not require a hapten-carrier bridge. In this hypothesis, Th2 cells behave as though they have two distinct receptors, i.e., one for Ig and one for antigen. It should be noted that Janeway's Th2 cells are responsible for the release of TRF and for the activation of particular clones of B cells. Our hypothesis about the IgE response may have some similarities to that of Janeway. However, a unique aspect of our hypothesis is that IgE-specific recognition sites on T cells or T cell factors are an $Fc_\epsilon R$ or their components rather than T cell receptors (for antigen). The possibility still remains that a portion of carrier-primed T cells may bear both antigen receptors and $Fc_\epsilon R$ and may be involved in the formation of both TRF and IgE-potentiating factor. However, it is more likely that the major source of IgE-potentiating factor is antigen-nonspecific $Fc_\epsilon R(+)$ T cells. Many investigators have suggested a possible role of Fc receptors on lymphocytes in antibody responses. Moretta *et al.* (30) reported that human T cells bearing $Fc_\gamma R$ have suppressive effects on the Ig-response, whereas T cells bearing $Fc_\mu R$ have helper effect. Gisler and Fridman (31) have shown that $Fc_\gamma R(+)$ T cells release an Ig-binding factor that regulates both IgM and IgG responses. Potentiation of the IgE response by IgE-potentiating factor, which is derived from $Fc_\epsilon R(+)$ T cells, may provide a unique example in which T cells with Fc receptors for a certain isotype are involved in isotype-specific regulation.

REFERENCES

1. Orr, T. S. C., and A. J. J. Blair. 1969. Potentiated reagin response to egg albumin and ovalbumin in *Nippostrongylus brasiliensis* infected rats. Life Sci. 8/II:1077.
2. Jarrett, E. E. E., and D. C. Stewart. 1972. Potentiation of rat reaginic (IgE) antibody by helminth infection: simultaneous potentiation of separate reagins. Immunology 23:749.
3. Bloch, K. J., J. L. Ohman, J. Waltin, and R. W. Cygan. 1973. Potentiated reagin response: initiation with minute doses of antigen and alum followed by infection with *Nippostrongylus brasiliensis.* J. Immunol. 110:197.
4. Jarrett, E. E. E., and A. Ferguson. 1974. Effect of T cell depletion on the potentiated reagin response. Nature 250:420.
5. Suemura, M., and K. Ishizaka. 1979. Potentiation of IgE response *in vitro* by T cells from rats infected with *Nippostrongylus brasiliensis.* J. Immunol. 123:918.
6. Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf. 1977. Methods in Immunology, W. A. Benjamin Inc., London. P. 135.
7. Eisen, H. N. 1964. Preparation of purified anti-2,4 dinitrophenyl antibodies. Method. Med. Res. 10:94.
8. Urban, J. F., Jr., T. Ishizaka, and K. Ishizaka. 1977. IgE-formation in the rat following infection with *Nippostrongylus brasiliensis.* III. Soluble factors for the generation of IgE-bearing lymphocytes. J. Immunol. 119:583.
9. Yodoi, J., and K. Ishizaka. 1979. Lymphocytes bearing Fc receptors for IgE. I. Presence of human and rat T lymphocytes with Fc_ϵ receptors. J. Immunol. 122:2577.
10. Urban, J. F., Jr., T. Ishizaka, and K. Ishizaka. 1978. IgE-B cell generating factor from lymph node cells of rats infected with *Nippostrongylus brasiliensis.* I. Source of IgE-B cell generating factor. J. Immunol. 121:192.
11. Suemura, M., J. F. Urban, Jr., and K. Ishizaka. 1978. Development of IgE-forming cells *in vitro* from rat mesenteric lymph node cells. J. Immunol. 121:2413.
12. Ishii, Y., H. Koshiba, H. Yamaoka, and K. Kikuchi. 1976. Rat T lymphocyte-specific antigens and their cross-reactivity with mouse T cells. J. Immunol. 117:497.
13. Conrad, D., and A. Froese. 1978. Characterization of the target cell receptor for IgE III. Properties of the receptor isolated from rat basophilic leukemia cells by affinity chromatography. J. Immunol. 120:429.
14. Ogilvie, G. M. 1964. Reagin-like antibodies in animals immune to helminth parasites. Nature 204:91.
15. Schlossman, S. F., and L. Hudson. 1973. Specific purification of lymphocyte population on a digestible immunosorbent. J. Immunol. 110:313.
16. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polyethylene tissue culture dishes coated with specifically purified anti-immunoglobulin. J. Immunol. Methods 15:47.
17. Julius, M. H., E. Simpson, and L. Herzenberg. 1973. A rapid method for the isolation of functional thymus derived lymphocytes. Eur. J. Immunol. 3:645.
18. Ishizaka, T., J. F. Urban, Jr., K. Takatsu, and K. Ishizaka. 1976. Immunoglobulin E synthesis in parasite infection. J. Allergy Clin. Immunol. 58:523.
19. Yodoi, J., and K. Ishizaka. 1979. Lymphocytes bearing Fc receptors for IgE. III. Transition of $Fc_\gamma R(+)$ cells to $Fc_\epsilon R(+)$ cells by IgE. J. Immunol. 123:2004.
20. Click, R. E., L. Benck, and B. J. Alten. 1972. Immune response *in vitro.* I. Culture conditions for antibody synthesis. Cell. Immunol. 3:264.
21. Gonzalez-Molina, A., and H. L. Spiegelberg. 1977. A subpopulation of normal human peripheral B lymphocytes that bind IgE. J. Clin. Invest. 59:616.
22. Ishizaka, T., J. F. Urban, Jr., and K. Ishizaka. 1976. IgE formation in the rat following infection with *Nippostrongylus brasiliensis.* I. Proliferation and differentiation of IgE-forming cells. Cell. Immunol. 22:248.
23. Yodoi, J., and K. Ishizaka. 1980. Lymphocytes bearing Fc receptors for IgE. IV. Formation of IgE-binding factor by rat T lymphocytes. J. Immunol. 124:1322.
24. Marrack, P. C., and J. W. Kappler. 1975. Antigen-specific and nonspecific mediators of T cell/B cell cooperation. I. Evidence of their production by different T cells. J. Immunol. 114:1116.
25. Marrack, P., and J. W. Kappler. 1976. Antigen-specific and nonspecific mediator of T cell/B cell cooperation. II. Two helper T cells distinguished by their antigen sensitivities. J. Immunol. 116:1373.
26. Tada, T., T. Takemori, K. Okumura, M. Nonaka, and T. Tokuhisa. 1978. Two distinct types of helper cells involved in the secondary antibody response. Independent and synergistic effects of Ia^- and Ia^+ helper T cells. J. Exp. Med. 147:446.
27. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sata, F. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T cell regulation of antibody response demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. J. Exp. Med. 114:330.
28. Woodland, R., and H. Cantor. 1978. Idiotype specific T helper cells are required to induce idiotype-positive B memory cells to secrete antibody. Eur. J. Immunol. 8:600.
29. Janeway, C. A., Jr. 1979. Idiotypic control: the expression of idiotypes and its regulation. *In* Regulation in the Immune System, Edited by E. Sercarz and A. Cunningham. In press.
30. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper. 1977. Functional analysis of two human T cells bearing receptors for IgM and IgG. J. Exp. Med. 146:184.
31. Gisler, R. H., and W. H. Fridman. 1975. Suppression of *in vitro* antibody synthesis by immunoglobulin binding factor. J. Exp. Med. 142:507.