Immunology 1993 **79** 580–586

Differentiation of membrane IgE$^+$ rat B cells into IgE-secreting cells

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Accepted for publication 16 March 1993

SUMMARY

Rat spleen cells were stimulated with pokeweed mitogen (PWM) and the IgM and IgE responses were assessed. An enrichment of the cell suspension with IgE-bearing cells before stimulation resulted in an increase in the number of IgE-secreting cells. A decrease of the number of IgE-secreting cells was found after depletion of IgE- or IgM-bearing cells, but not those bearing IgD molecules on their membranes, before stimulation. Moreover, the stimulation of membrane IgE on B cells with anti-IgE antibodies was shown to increase the number of IgE-secreting cells after PWM-induced differentiation *in vitro*. *In vivo*, it was also observed that a single injection of anti-IgE antibodies can induce the differentiation of IgE-secreting cells. These results demonstrate the presence of IgE$^+$–IgM$^+$–IgD$^-$ B cells in the rat that are responsive to PWM-induced differentiation into IgE-secreting cells. They indicate a pre-commitment of these cells at a stage where they still express IgM on their surface. IgE molecules on the cell membranes play a role in their differentiation.

INTRODUCTION

Mature B cells express IgM or co-express IgM and IgD, involving a differential processing of long nuclear transcripts. Then VDJ–Cμ–Cδ transcripts can be differentially spliced to give either μ mRNA or δ mRNA.[1] During their differentiation, B cells may switch to the expression of other isotypes: IgG, IgA or IgE. Analysis of the H-chain genes in cells representing later stages in B-cell differentiation, namely myeloma cells and hybridomas, suggests that switching is accomplished by rearrangement of the expressed C region gene, with deletion of the intervening DNA.[2] This model implies that cells may not simultaneously express multiple isotypes excluding IgM and IgD.

The presence of IgM with IgG, IgE or IgA molecules, however, has been reported in normal lymphocytes[3-8] and murine or human B-cell lines.[9-14] Moreover, recent observations in our laboratory have shown the presence in the rat of a B-cell pool co-synthetizing membrane IgM and IgE molecules.[15] Alternative processing of large nuclear transcripts[16] or trans-splicing of sterile C_H transcripts[17] have been suggested to be responsible for these co-expressions. These multiple isotype expressions are thought to occur physiologically during the differentiation.[18]

The presence of cells expressing IgM with IgG, IgA or IgE raises the following questions: are these cells (pre)committed to the production of the isotype expressed with IgM and what is the role of these molecules on the cell membranes? In mice, it was shown that pre-B cells expressing sterile Cγ2b transcripts preferentially express IgG2b molecules when transformed with the murine Abelson leukaemia virus.[18]

To clarify the relationship between sIgE-expressing B cells and the fully differentiated IgE-secreting cells in normal animals, we have investigated the IgE production of spleen cells stimulated with pokeweed nitrogen (PWM) after sorting of IgE-, IgM-, and IgD-expressing cells. Isotype-specific monoclonal antibodies (mAb) and F(ab')$_2$ fragments were used in the sorting processes. The results show that the few B cells expressing sIgE are indeed involved in the differentiation of IgE-secreting cells and that they also express IgM but few or no IgD molecules. Moreover, on these cells, IgE molecules may act as antigen receptors for activation.

Abbreviations: FCM, flow cytometry; mAb, monoclonal antibody; N.b., *Nippostrongylus brasiliensis*, PWM, pokeweed mitogen; sIg, surface immunoglobulin.

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MATERIALS AND METHODS

Rats, antibodies and F(ab')$_2$ fragments

Conventional 8–10-week-old inbred LOU/C.Igκ-1b rats were used; they were bred in our laboratory.

The mouse mAb specific for the rat Igκ light chain (MARK-1: γ1, κ and MARK-3: γ1, κ) and the rat isotypes μ (MARM-7 and MARM-4: γ1,κ), δ (MARD-3: γ1, κ) and ε (MARE-1: γ1, κ) have been described elsewhere.[19] The LO-RK1b-2 is a rat mAb (γ2a, κ), specific for the rat Igκ-1b light chain.[20] These antibodies were produced in ascitic fluids and purified by affinity chromatography on Sepharose-4B–rat Ig isotype columns.[21] They can be commercially obtained from Serotec (Bicester, U.K.) or Zymed Labs (San Francisco, CA). The mouse IgG1 mAb against DNP (BO8401H5) and the mouse IgG1 myeloma protein (BO2) were kindly given by J. Van Snick and G. Burtonboy (UCL, Brussels, Belgium).

Aggregation of rat IgE molecules from the IR2 immunocytoma[22] was carried out by reaction with 30 times molar excess of dimethylsuberimidate (Sigma, St Louis, MO) for 2 hr, 30°. The resulting aggregates showed a molecular weight (MW) superior to 500,000.

A specific polyclonal antibody against rat IgE was obtained by immunization of a goat with rat monoclonal IgE from IR2, IR162, IR410, IR962 and IR1016 rat immunocytomas.[22]

Rabbit serum against rat IgM was obtained by injection of rat monoclonal IgM from IR968, IR202 and IR990 immunocytoma cells,[23] and rabbit anti-rat IgD by injection of monoclonal IgD from IR731 immunocytoma cells.[24] Cross-reactivities of these sera against other rat isotypes were eliminated by passage through immunoaffinity columns of Sepharose-4B–rat Ig isotypes other than those used for immunization. These sera were then immunopurified on Sepharose-4B–rat Ig isotype corresponding to the antigenic specificity, and the monospecificity was verified by radial immunodiffusion. $(NH_4)_2SO_4$ precipitation of normal goat serum was used to prepare semi-purified normal goat IgG.

The MARE-1 mAb was dialysed against 0·1 M acetate buffer, pH 5·5 and digested with papain (Sigma) at 20:1 protein-to-enzyme ratio for 27 hr at 37°. The resulting digest was passed on a DEAE–Sepharose gel (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) followed by immunoaffinity chromatography on Sepharose-4B (Pharmacia)–LO-MK-2 mAb (rat anti-mouse κ-light chain) to retain Igκ-containing fragments and on Sepharose-4B–LO-MG1-2 mAb (rat anti-mouse IgG1 heavy chain) to remove any residual undigested IgG and Fc fragments. SDS–PAGE of the F(ab′)$_2$ fragments showed a unique band with a MW of 114,000.

The MARE-1 mAb and its F(ab′)$_2$ fragments were biotinylated. In brief, proteins were dialysed in 0·1 M carbonate–bicarbonate buffer, pH 8·0. The *N*-hydroxysuccinemido-biotin was dissolved in dimethylformamide (1 mg/ml) and was added to dialysed samples (20 μl for 1 mg proteins). The mixture was incubated at room temperature for 10 min and then 10 μl NH_4Cl 1 M/mg protein was added. The protein was dialysed overnight against phosphate-buffered saline (PBS), pH 7·2.

The MARM-7 and MARD-3 (5 mg/ml, 0·1 M carbonate–bicarbonate, pH 9·5) were stained by mixing at room temperature for 2 hr, with fluorescein isothiocyanate (FITC) (45 μg/ml proteins). FITC-stained proteins were separated from free FITC on a Sepharose-G25 column.

The MARK-1, MARK-3 and LO-RK1b-2 mAb were conjugated to horseradish peroxidase (HRP) as decribed by Nakane and Kawaoi.[25]

Removing cytophilic IgE

A pellet of 2×10^8 spleen cells was mixed (45 seconds on ice) with 1 ml of citrate buffer (pH 4·0) and washed in PBS.[26] In our experience, such a treatment removed 95% of the sIgE from lymphocytes of rats infected with *Nippostrongylus brasiliensis* (N.b.) (gift from Union Chimique Belge, Belgium) without affecting sIgM and sIgD expression.

Separation of B-cell subsets acccording to sIg expression and immunofluorescence analysis

Spleen cell suspensions were used in the experiments. The cells expressing sIgM were depleted by incubation in Petri dishes (90 mm: Nunc, U.K.) precoated overnight at 4° with MARM-7 or MARD-3 mAb (5 μg/ml in borate buffer, pH 9·5). Free binding sites were blocked for 1 hr at 37° with culture medium before addition of 10^7 cells/dish for 2 hr at 37°. Non-adherent cells were reincubated on new Petri dishes under the same conditions. Flow cytometry (FCM) analysis of the depleted cells was performed (staining of the cells with the MARM-7–FITC or MARD-3–FITC mAb) with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) using a single argon laser and logarithmic intensity. Dead cells and non-lymphoid cells were excluded from the analysis based on forward and sideway scatter.

Acid buffer-treated spleen cell suspensions were depleted of sIgE-expressing cells by mixing with MARE-1-coated magnetic beads (Dynabeads, Dynal AS, Oslo, Norway), according to the Dynal protocol, using a beads/stained cells ratio of 40:1.

Depletion and enrichment of sIgE$^+$ cells were obtained in one step using the MACS system (Miltenyl Biotec, Germany): acid buffer-treated cells were labelled sequentially with biotinylated F(ab′)$_2$ fragments of the MARE-1 mAb (5 μg/ml in cold culture medium for 30 min at 0°), with avidin (AV)–FITC [Becton Dickinson; 200 μl for 2×10^8 cells, for 15 min, 0° in PBS–BSA (bovine serum albumin) 1%] and with MACS biotinylated microbeads (10 μl for 2×10^8 cells, 5 min, 0° in PBS–BSA 1%). Cells were passed through the A1 MACS separation column. Labelled cells stuck to the magnetizable matrix of the column and the unlabelled cells flowed through. The magnetic fraction was eluted after taking away the magnetic field. Direct FCM control of the labelled cells was possible through the biotin AV–FITC bridge between particles and the biotin mAb.

Cell culture and stimulation

Cells were suspended in Dulbecco's minimal essential medium (DMEM) (Gibco, Paisley, U.K.) supplemented with sodium pyruvate 2%, non-essential amino acid 1%, L-glutamine 1%, gentamycin (Gibco), 5% horse serum and 5% foetal calf serum (FCS). They were cultured for 6 days in 24-well plates (10^6 cells/well in 1 ml, 37°, 5% CO_2).

Induction of IgE-secreting cells was obtained by addition, at day 1, of 10 μl/well PWM (Gibco) and of 200 μl of a concanavalin A (Con A)-stimulated spleen cell supernatant (5×10^6 cells/ml were incubated for 24 hr at 37° with 10 μg/ml Con A, after which 40 μg/ml Methyl α-D-Mannopyrannoside was added).

ELISA

IgE and IgM levels in the culture supernatant were determined by ELISA. Microtitre plates (Flow Laboratories, Rockville, MD) were coated (overnight, 4°, 5 μg/ml in borate buffer pH 9·5) with 100 μl MARE-1 or MARM-4 mAb/well.

The plates were washed, and the remaining active sites were blocked for 1 hr with PBS–0·5% gelatin. After washing with PBS–0·1% Tween-20, samples (100 μl/well) were added, and plates were incubated at 37° for 2 hr. Wells were washed and 100 μl of peroxidase-labelled MARK-1 (0·5 μg/ml) was added to each well. After 2 hr at 37°, the plates were washed three times with PBS–0·1% Tween-20. Peroxidase was revealed by the addition of 100 μl/well of ortho-phenylene-diamine [(OPD) 0·4 mg/ml; Sigma] in a citrate–phosphate buffer (pH 5·0) with H_2O_2 30% (1/10,000) for 15 min in the dark. Reaction was stopped with the addition of 50 μl/well of 2 M H_2SO_4.

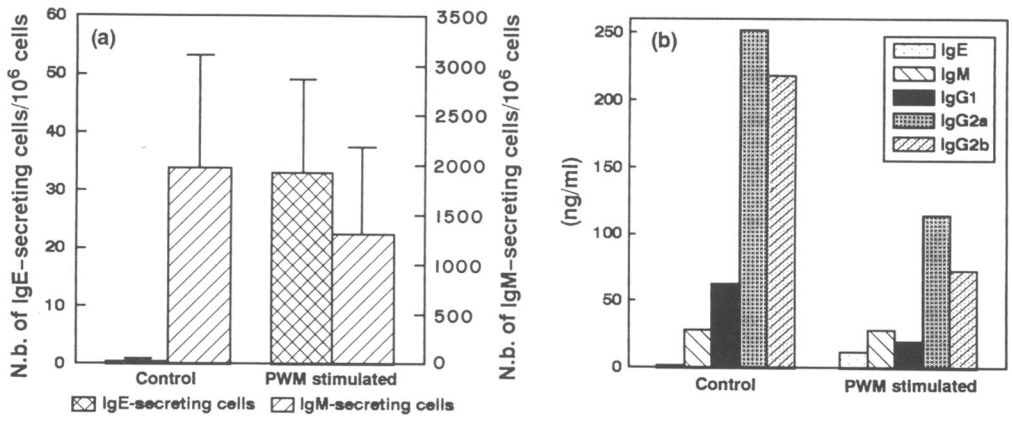

Figure 1. *In vitro* culture of rat spleen cells stimulated with PWM for 5 days. (a) Numbers of IgE- and IgM-secreting cells after culture. Results are presented as mean ± SD of six independent experiments. (b) Supernatant immunoglobulins after culture; mean of two observations.

The standard curve was constructed with IgE from the IR2 immunocytoma[22] purified by affinity chromatography and titrated (Bradford method).

ELISA-spot

This assay was developed to count the IgE- and IgM-secreting cells. Nitrocellulose (Schleisher and Shuller, Germany) discs (diameter 1 cm) were incubated overnight at 4° with 5 μg/ml MARE-1 or MARM-4 mAb in a borate buffer (pH 9·5). After washing in PBS, they were left for 2 hr at room temperature in culture medium containing 5% horse serum and 5% FCS, washed again and left to dry at room temperature.

To count IgE-secreting cells, 10^6 cells in 40 μl of culture medium were put on nitrocellulose discs inside the wells of a 24-well culture plate. The plates were incubated for 20 hr at 37°, 5% CO_2. To count IgM-secreting cells, a suspension of $0{\cdot}5 \times 10^5$ cells in 40 μl was used.

After incubation, papers (in the wells) were washed twice with PBS and four times with PBS–0·1% Tween-20. Ig molecules bound to the papers were revealed with a mixture of peroxidase-labelled MARK-1, MARK-3 and LO-RK1b-2 mAb (1 μg/ml of each antibody, 0·5 ml/well, 37° for 3 hr). The papers were washed four times with PBS–0·1% Tween-20 and once with PBS, and 0·5 ml/well of a mixture of 4-chloronaphthol (Sigma, 4 mg/ml first dissolved in ethanol) and H_2O_2 30% (1/1000) in PBS were added. Black spots appeared within 2 hr, at room temperature. Papers were washed with water and dried before analysis. Counting was performed with a squared objective of a stereoscopic microscope (magnification ×40). Student's *t*-test was used to analyse the data.

RESULTS

PWM-induced differentiation of IgE-secreting cells

Fresh spleen cells contained less than one IgE-secreting cell/10^6 cells before and after a 6-day culture. Addition of PWM, at day 1 of the culture, induced the appearance of 20–40 IgE-secreting cells/10^6 cells. The number of IgM-secreting cells was not affected by the addition of PWM (Fig. 1a). The titration of immunoglobulins in the culture supernatants showed that the IgE level increased under PWM stimulation, the IgM level did not change and the IgG1, IgG2a, IgG2b subclasses were less represented (Fig. 1b).

Separation of lymphocytes based on sIgM, sIgD and sIgE expressions

Panning procedures were applied for depletion of IgM- and IgD-bearing cells before PWM-induced differentiation. A sus-

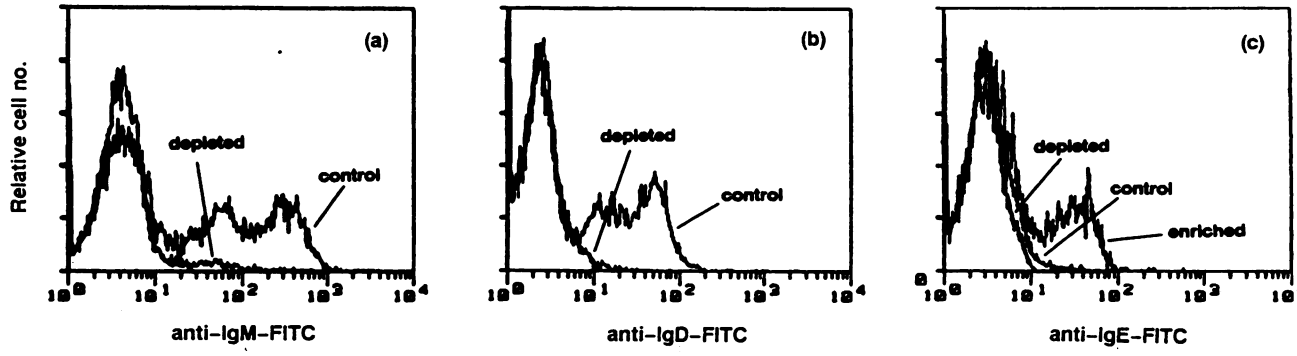

Figure 2. Depletion of sIgM-expressing cells (a) and sIgD-expressing cells (b) from rat spleen cells by a two-step panning with anti-Ig mAb. (c) Depletion and enrichment of sIgE-expressing cells using the MACS system, with $F(ab')_2$ fragments from the anti-IgE mAb.

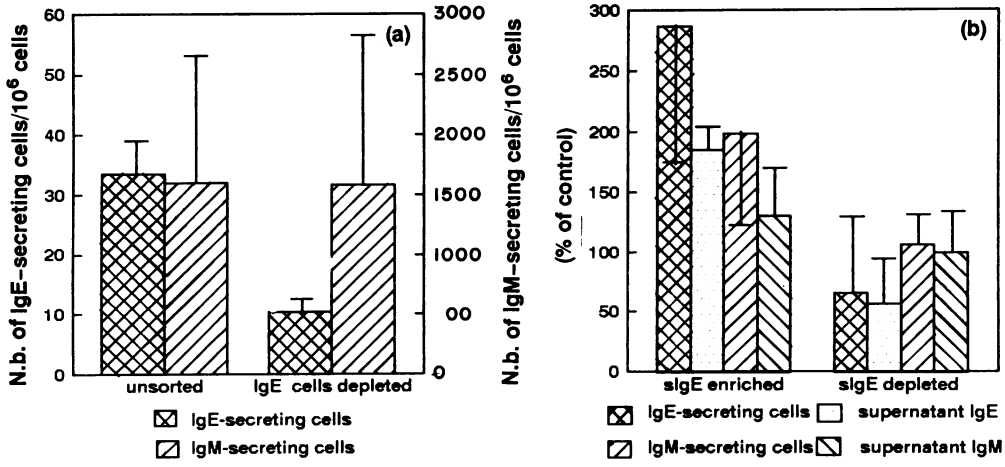

Figure 3. Spleen cells were sorted on the basis of surface immunoglobulin and *in vitro* stimulated with PWM for 5 days. After culture, IgE- and IgM-secreting cells were counted and supernatant IgE and IgM were titrated. (a) sIgE$^+$-depleted cells using the anti-IgE mAb-coated Dynabeads. (b) sIgE$^+$ depletion and enrichment using the MACS system with F(ab')$_2$ fragments from the anti-IgE mAb. Control values are from the same cell preparation, not subjected to the MACS separation but stimulated in the same way. These control values are: IgE, 10·7 ± 2·2 IgE-secreting cells/10^6 cells and 16 ± 4·3 ng/ml in the supernatant; IgM, 470 ± 109 IgM-secreting cells/10^6 cells and 30 ± 8 ng/ml in the supernatant. Results are means ± SD of four independent experiments.

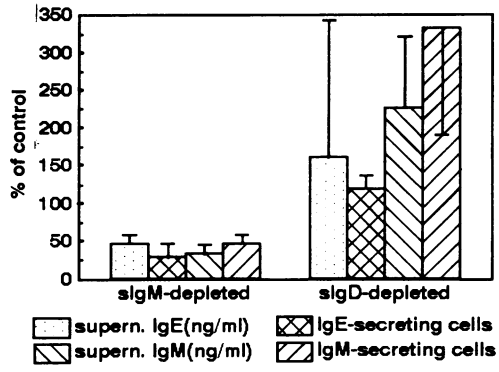

Figure 4. Spleen cells were depleted of sIgM$^+$ and sIgD$^+$ cells by a two-step panning with anti-Ig mAb and *in vitro* stimulated with PWM for 5 days. The numbers of IgE- and IgM-secreting cells and the supernatant IgE and IgM molecules were assessed. Controls were stimulated but unsorted cells. The control values are: IgE, 40 ± 10 IgE-secreting cells and 15 ± 5 ng/ml in the supernatant; IgM, 2000 ± 100 IgM-secreting cells and 25 ± 5 ng/ml in the supernatant. Results are expressed as means ± SD of three independent experiments.

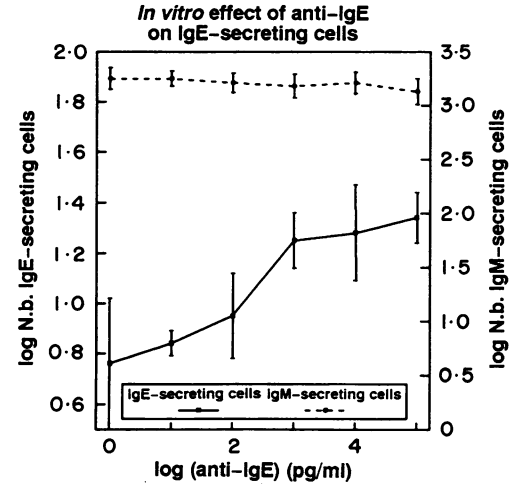

Figure 5. Spleen cells were *in vitro* stimulated with PWM and increasing concentrations of goat serum against rat IgE were added. After a 5-day period, the numbers of IgE- and IgM-secreting cells were established with the ELISA-spot assay. Results are means ± SD of four experiments.

pension of spleen cells was incubated on anti-IgM or anti-IgD mAb-coated Petri dishes and the cells remaining in the supernatant were collected. As illustrated in Fig. 2a, b, FCM analysis of the depleted cell populations showed that > 85% of the sIgM-expressing cells, and > 95% of the sIgD-expressing cells were removed by this process.

Dynabeads coated with anti-IgE mAb were mixed to acid buffer-treated spleen cells to remove the sIgE$^+$ cells before PWM induction. One of 10^4 living cells reacted with the beads providing rosettes (more than two beads/cell), and no rosette was found when using Dynabeads coated with mouse anti-DNP mAb of the same isotype. Spleen cells reacting with the beads could not be analysed because they died within a few days in culture. It was not possible to remove beads from the rosetted cells.

In the same way, acid buffer-treated spleen cells were also sequentially stained with biotin-conjugated F(ab')$_2$ anti-IgE mAb, AV-FITC, biotin-conjugated MACS microbeads, and separated with the MACS system according to the presence of the immunomagnetic particles. FCM analysis suggested that 10–30% of sIgE-expressing cells were obtained while starting with undetectable levels of these cells (Fig. 2c).

A May–Grünwald–Giemsa staining carried out immediately after the sorting process showed that enrichment of IgE$^+$ B cells induces a 20–70% increase of the number of blasts (data not shown).

Table 1. *In vivo* effect of anti-IgE antibodies on IgE production*

Injection of	Exp. 1†	Exp. 2‡
PBS	<1§	<1
Goat anti-IgE Ab	39±18·6	ND
Normal goat IgG	7±9·1	ND
MARE-1	29·6±3·2	16·8±4·1
B8401H5	14·1±1·2	ND
BO2	ND	6·7±2·7

* Adult LOU/C rats were i.p. injected with 1 mg of immunopurified anti-rat IgE goat serum, or with 1 mg of mouse IgG1 mAb against rat IgE (MARE-1). Controls were performed with injections of 1 mg of goat IgG or mouse IgG1 (B8401H5 and BO2). Five days later, spleen cells were assessed for the number of IgE-secreting cells, using the ELISA-spot assay.

† Mean±SD from four rats/treatment.

‡ Mean±SD from three rats/treatment.

§ Number of IgE-secreting cells/10^6 spleen cells.

Effect of depletion or enrichment of IgE-bearing cells on IgE production

Spleen cells depleted of IgE-bearing cells with magnetic Dynabeads were stimulated *in vitro* by PWM and the numbers of IgE- and IgM-secreting cells were assessed 5 days later. Speen cells treated similarly with B8401H5-coated Dynabeads were used as controls. This treatment resulted in a significant decrease (Student's *t*-test, $P<0·05$) of the number of IgE-secreting cells after *in vitro* differentiation, without affecting the number of IgM-secreting cells (Fig. 3A).

A very low and not significant reduction of the IgE expression was observed after IgE^+ B cells depletion through the MACS system. A considerable increase of both IgE-secreting cells and produced IgE was observed when enriched IgE-bearing cell populations were stimulated by PWM ($P<0·05$). No statistical difference in IgM secretion was found between the two populations (IgE^+ and IgE^-).

Effect of depleting IgM- or IgD-bearing cells on IgM and IgE production

To examine whether the precursors of IgE producers also expressed cell-surface IgM and IgD, the IgM^+ and IgD^+ subpopulations of B cells were depleted before stimulation with PWM, by a two-step panning procedure. As shown in Fig. 4, a significant reduction in IgE production was observed in sIgM-expressing cell-depleted populations ($P<0·05$ for supernatant IgE and $P<0·01$ for IgE-secreting cells). In contrast, no similar conclusion could be obtained after sIgD-expressing cell depletion, suggesting that precursors of IgE-secreting cells do express sIgM, but not necessarily sIgD. The IgM production was increased after PWM stimulation of sIgD-expressing cell-depleted populations and reduced if $sIgM^+$ B cells were removed ($P<0·05$). This shows that IgM-secreting cells in PWM-induced populations expressed sIgM but few or no sIgD molecules before stimulation.

In vitro and *in vivo* enhancement of IgE production with anti-IgE antibodies

Surface immunoglobulins were stimulated during spleen cell cultures to assess their role in PWM-induced differentiation into IgE-secreting cells. The number of IgE-secreting cells was not affected with 50 μg/ml anti-IgM or anti-IgD antibodies in the culture medium using rabbit polyclonal or mouse monoclonal (MARM-7, MARD-3) antibodies. Addition of aggregated IgE molecules or MARE-1 mAb has no effect in this system while purified goat anti-IgE antibodies induced a threefold increase in the number of IgE-secreting cells. As shown in Fig. 5, the goat anti-IgE antibodies affected the IgE production in a dose-response relationship. Maximal effect was already seen when 1 ng/ml anti-IgE antibodies was used. This treatment does not affect IgM production.

In view of these results, the *in vivo* effect of anti-IgE antibodies was also tested. A single administration of MARE-1 mAb or goat anti-rat IgE antibodies induced the appearance of a small but significant number of IgE-secreting cells in the spleen, 5 days after treatment. Injection of normal goat IgG or mouse IgG1 resulted also in a small IgE response, significantly lower than with anti-IgE antibodies ($P<0·05$). Unstimulated rats did not show detectable IgE-secreting cells (Table 1).

DISCUSSION

Culture of normal rat lymphocytes with PWM provides a short-term differentiation in which the lymphokine products of the activated T cells indirectly promote B-cell differentiation.[27]

We have chosen to study the IgE lineage for its importance in immediate hypersensitivity[28] and its role in immunity against parasites.[29] Moreover, its very low background secretion in normal animals and its high production *in vitro* after PWM stimulation make the study of IgE lineage easier than that of other isotypes.

The mechanisms of the induction of IgE synthesis (see ref. 30 for review) are not fully defined. Interleukin-4 (IL-4) alone can induce C_ε germ-line transcription in resting B cells. Such a pre-switch induction of germ-line C_H transcripts by mitogens or interleukins is now well documented for all the isotype subclasses.[17,18,31-38] Moreover, co-expressions on memory B cells after *in vivo* priming have been reported[8] showing that IgM–IgG-expresing cells represent an intermediate stage during the differentiation.

Our previous observations of IgM–IgE co-expressing B cells in the rat showed that both Ig isotypes in these cells are really synthetized and do not correspond only to trace amount.[15] Numerous studies reporting such co-expressions raise the question of the developmental stage of these cells. Because of the expression of IgM, clearly these IgE^+ IgM^+ co-expressing cells have not deleted their intervening DNA between the C_ε locus and VDJ segments. Thus, these cells are not genetically committed to the expression of one isotype. However, some aforementioned results and the present report show that B cells co-expressing IgM with another isotype (IgG, IgA or IgE) preferentially produce this isotype after stimulation. We found indeed a correlation between the depletion of $sIgE^+$ and $sIgM^+$B cells before PWM induction and the subsequent

decrease of IgE production. A similar correlation was obtained with the sIgE$^+$ B-cell enrichment procedure leading to an increase in IgE production. This does not imply that such B cells are compulsorily committed to the production of IgE but suggests that there could be a stage during the differentiation when B cells are prepared for the commitment. These pre-committed B cells can express IgE in their membrane but have not yet lost the IgM expression.

We do not know whether these IgE$^+$ IgM$^+$ B cells come from a previous IgE immune response, thus being memory B cells, of if they arise spontaneously. It was reported that IgG subclasses or IgE isotypes can be expressed on the membrane of neonatal liver B cells from mice[39] or rat[40] and also athymic nu/nu mice.[39] Peyer's patches from germ-free rats were shown to contain more than 15% of membrane IgE$^+$ B cells.[4] In humans, it was shown that some neonatal lymphocytes from cord blood may differentiate into IgE-secreting cells after IL-4 stimulation.[41] Taken together, these results suggest that B cells can synthetize isotypes other than IgM–IgD without the requirement of antigenic stimulation. Thus, IgE$^+$ IgM$^+$ B cells found in conventional rats are not necessarily coming from previous exogen antigenic stimulation, and it could represent a pre-existing B-cell subpopulation pre-committed to the production of IgE.

After depletion of the sIgE-expressing cells, the IgE response of the cell population was reduced, but not totally suppressed, suggesting that some sIgE$^+$ B cells escaped from sorting. Moreover, the possibility is not excluded that some IgE$^+$ IgM$^+$ IgD$^+$ B cells can also respond to PWM induction, but in a lower percentage or to a weaker extent than the IgE$^+$ B cells. It is conceivable that IgE$^-$ IgM$^+$ IgD$^+$ B cells correspond to a less mature stage than IgE$^+$ B cells. Besides, we cannot exclude the possibility that there are a few resting IgE$^+$ IgM$^-$ post-switch B cells among normal rat B cells (200 ng/ml serum IgE are found in these rats). These IgE$^+$ IgM$^-$ B cells could be subject to PWM induction and participate in the IgE production, explaining why depleting more than 85% of the IgM$^+$ cells results in only a 50–70% reduction of IgE secretion.

The regulation of IgE production by IgE or anti-IgE molecules is still not well understood. It appears that the induction of auto-anti-IgE antibodies in the rat, with anti-isotype or anti-idiotype specificity, can both enhance or reduce the serum IgE concentrations, depending on the strain.[42] Anti-IgE antibodies can inhibit the sCD23 release by bridging cytophilic IgE, resulting in an inhibition of the B-cell multiplication,[43] but other anti-IgE antibodies could inhibit IgE binding to the membrane form of CD23, resulting in opposite effects. An increase in serum IgE following anti-IgE injection could also be explained by direct stimulation of membrane IgE$^+$ precursor B cells.

We favour the last hypothesis as the MARE-1 mAb, which does not interfere with the IgE binding on the CD23, induced an *in vivo* differentiation of IgE-secreting cells. On the other hand, aggregated IgE failed to increase the *in vitro* PWM-induced differentiation. Moreover, Mizuguchi *et al.*[44] have shown that membrane immunoglobulins other than IgM and IgD act as signal transmission molecules.

In conclusion, we consider that IgE$^+$ IgM$^+$ B cells in normal rats are direct precursors of IgE-secreting cells and suggest that membrane IgE may serve as a triggering signal leading to the expansion and maturation of this cell population.

ACKNOWLEDGMENTS

We are grateful to Mrs F. Bolle and L. De Greef for secretarial assistance. This work was supported by the 'Fonds de la Recherche Scientifique et Médicale' (Belgium) and 'Institut pour l'Encouragement de la Recherche dans l'Industrie et l'Agriculture' (Belgium). H.B. is staff member of the Biotechnology Division of the Commission of the European Communities.

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