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Selective enhancement of human IgE production *in vitro* by synergy of pokeweed mitogen and mercuric chloride

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SUMMARY

IgE production *in vitro* was investigated in cultures of human peripheral blood mononuclear cells (MNC) from non-atopic donors with pokeweed mitogen (PWM), mercuric chloride ($HgCl_2$), or both. PWM alone induced a few IgE immunoglobulin secreting cells (ISC) detected by reverse plaque forming cells (PFC) and many IgG, IgM, and IgA PFC. $HgCl_2$ alone failed to produce significant numbers of ISC of any class. PWM plus $HgCl_2$ caused a selective increase of IgE PFC without affecting IgG, IgM, and IgA PFC. Co-cultures of B cells plus mitomycin C (MMC) treated T cells stimulated by PWM alone produced more IgG, IgM, IgA and IgE PFC than those of B cells plus T cells. However, PWM plus $HgCl_2$ produced significantly more IgE PFC selectively in those cultures. This effect was observed in the cells of most of the donors, but a few donors showed different responses.

Keywords pokeweed mitogen mercuric chloride IgE production mononuclear cells

INTRODUCTION

Recently, many laboratories have reported *in vitro* systems of human IgE production, both in patients with high serum IgE (Patterson *et al.*, 1975; 1976; Buckley & Becker, 1978; Ghory *et al.*, 1980; Sampson & Buckley, 1981) and in atopic patients (Tjio, Hull & Gleich, 1979; Ureña *et al.*, 1979 Fiser & Buckley, 1979; Saxon, Morrow & Stevens, 1980b; Romagnani *et al.*, 1980a, 1983a, 1982b; Turner, Holt & Holt, 1981; Holt, Turner & Holt, 1981) and normal donors (Saxon & Stevens, 1979; Saxon, Kaplan & Stevens, 1980a; Pryjma *et al.*, 1980; Nonaka *et al.*, 1981; Zuraw *et al.*, 1981; Nagai *et al.*, 1982). Some workers have succeeded in enhancing IgE production by pokeweed mitogen (PWM) in cells from normal donors (Saxon & Stevens, 1979; Pryjma *et al.*, 1980; Zuraw *et al.*, 1981). Moreover, synergy of PWM plus several agents enhanced IgE responses (Nonaka *et al.*, 1981; Nagai *et al.*, 1982).

In animals, IgE potentiating factor, one of the IgE binding factors, was found in the culture supernatant of T cells from parasite infected rats (Suemura & Ishizaka, 1979) or when activated with 10 μg/ml of Con A (Yodoi, Hirashima & Ishizaka, 1981) and in the serum of *Bordetella pertussis* vaccine (BP) treated rats (Hirashima, Yodoi & Ishizaka, 1981). Katz *et al.* (1979) found enhancing factors of allergy (EFA) in mice. Therefore, it is very tempting to look for factors or mitogens which enhance human IgE production *in vitro*. Prouvost-Danon *et al.* (1981) reported that mercuric chloride ($HgCl_2$) induced a striking increase of total serum IgE in Brown–Norway rats. We tested whether $HgCl_2$ either alone or with PWM could potentiate *in vitro* IgE production in cells from normal donors.

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MATERIALS AND METHODS

Cell donors. Blood was obtained from non-atopic donors aged 24–32 years. All donors had serum IgE levels below 100 iu/ml. In some experiments, blood from a patient with bronchial asthma who had serum IgE over 4,000 iu/ml, and spleen cells from a patient with hereditary spherocytosis, who had normal serum IgG, IgM, IgA and IgE levels, were used.

Lymphocyte preparation and separation procedures. Heparinized blood was drawn by venipuncture and diluted 1:1 with phosphate-buffered saline (PBS), then underlayered with Ficoll-Paque (Pharmacia, Fine Chemical AB, Uppsala, Sweden). The tubes were centrifuged for 30 min at 400*g* at room temperature, and the interface cells were collected as mononuclear cells (MNC) and washed three times in PBS (Böyum, 1968). T and B lymphocyte fractions were separated by rosetting with sheep red blood cells (SRBC) treated with 2-aminoethyl-isothiouronium bromide (AET) followed by Ficoll-Paque centrifugation (Saxon, Feldhaus & Robins, 1976). Separated T cell fractions had greater than 90% SRBC rosette forming cells and less than 2% membrane immunoglobulin bearing cells. The B cell fractions contained less than 5% rosette forming cells, 50–60% membrane bearing cells, and 20–30% monocytes as determined by non-specific esterase staining. Neither T cells nor B cells alone produced significant ISC of any class when stimulated with PWM. Some T cells were incubated with 50 μg/ml mitomycin C (MMC, Kyowa Hakko, Japan) for 30 min at 37°C and washed three times in PBS.

Spleen cells were obtained immediately after splenectomy. The spleen was gently sliced in an ice bath under sterile condition in Eagle's minimum essential medium (MEM, Nissui Seiyaku Co. Ltd., Japan) supplemented with 10 mM *N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES, Nakarai Chemicals, Ltd., Japan) and passed through a stainless steel wire sieve. Large clumps of cells and tissue fragments were allowed to settle for 5 min, and the supernatant cell suspension was recovered and centrifuged on a Ficoll-Paque density gradient. The MNC recovered at the interface were collected and washed three times in MEM.

Mitogens. PWM (GIBCO, Grand Island, New York, USA) was used. $HgCl_2$ (Nakarai) was dissolved in RPMI 1640 (Nissui) and sterilized by filtration through a 0·22 μm millipore filter (Millipore Corp., Bedford, Massachusetts, USA).

Lymphocyte cultures. Each cell fraction was suspended in RPMI 1640 buffered with $NaHCO_3$ and supplemented with 20% heat-inactivated fetal calf serum (FCS, GIBCO), 4 mM L-glutamine (Nakarai), 50 u/ml penicillin (Meiji Seika Kaisha, Ltd., Japan), 50 μg/ml streptomycin (Meiji). Cell suspensions (0·2 ml) of unfractionated MNC (2×10^5), B cells ($0{\cdot}4 \times 10^5$) plus T cells ($1{\cdot}6 \times 10^5$), and B cells $0{\cdot}4 \times 10^5$) plus MMC treated T cells ($1{\cdot}6/10^5$) were cultured with various concentrations of PWM, $HgCl_2$, or both in round bottomed microtitre plates (Flow Laboratories, Hamden, Connecticut, USA) in a humidified atmosphere at 37°C with 5% Co_2, 95% air for 5–7 days.

Antisera. Rabbit anti human-IgG, IgM, IgA and IgE sera (Behringwerke AG, Marburg, W. Germany) were used. The specificity of each was confirmed by blocking tests (Rector *et al.*, 1980).

Assay for immunoglobulin secreting cells (ISC). ISC were counted by modified reverse protein A (Pharmacia) plaque assay (Gronowicz, Coutinho & Melchers, 1976). Briefly, cultured cells were harvested and washed once in MEM, then washed once in FCS, which adsorbed the cytophilic Ig and again washed in MEM, then suspended in MEM. Cell suspensions (0·1 ml) were mixed with 25 μl lyophilized guinea-pig complement, 25 μl rabbit antiserum and 50 μl 20% protein A coated SRBC and placed in Cunningham chambers (Takahashi Giken Glass, Co. Ltd., Japan). Plaque forming cells (PFC) were scored under ×40 magnification after incubation at 37°C for 6–12 h. The optimal final concentrations of the antisera were 1:160 for anti-IgG, IgM and IgA and 1:320 for anti-IgE. Because the day of maximum response varied with each donor, plaques were detected on day 5–7, and the peak responses were compared. Data of IgG, IgM and IgA ISC were expressed as the number of PFC/2×10^4 cells initially cultured ± s.d. and IgE ISC as PFC/2×10^5 cells initially cultured ± s.d.

RESULTS

Failure of ISC induction by $HgCl_2$

Unfractionated MNC of normal donors were cultured with various concentrations of $HgCl_2$ and ISC were detected. As controls, MNC were cultured with PWM 10 μl/ml or without mitogen. As shown in Fig. 1, $HgCl_2$ alone failed to induce any significant ISC at any concentration, while PWM produced many IgG PFC and a few IgE PFC. Though not shown, many IgM and IgA PFC were also induced by PWM. Toxic levels of $HgCl_2$ decreased IgG PFC.

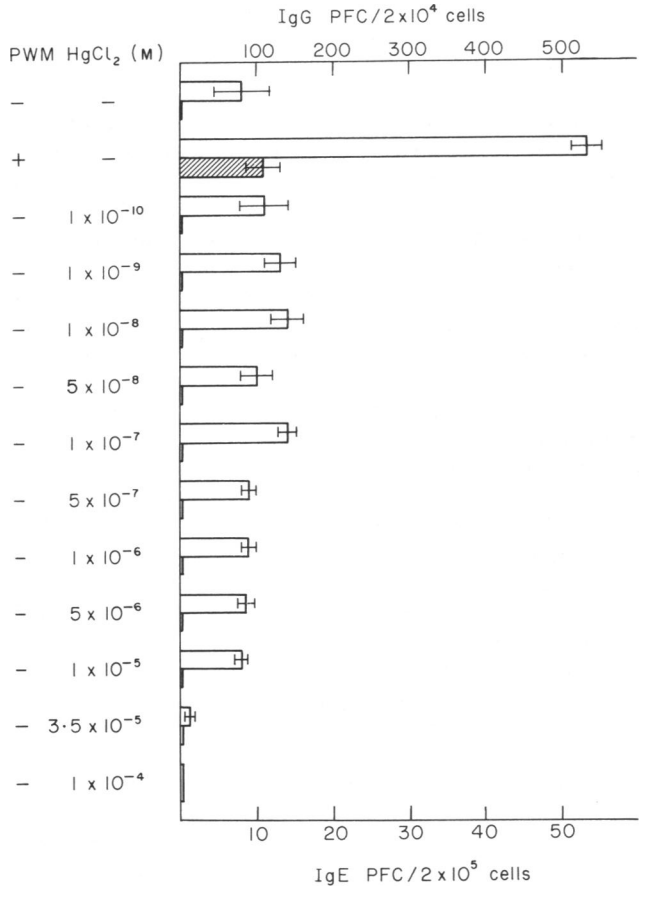

Fig. 1. Induction of ISC in cultures with PWM 10 μl/ml, various concentrations of $HgCl_2$ and without mitogen. Peak PFC of four classes were detected on day 7. Only IgG (□) and IgE (■) PFC are shown.

Synergy of PWM and $HgCl_2$ in induction of ISC of various donors

We next combined PWM and $HgCl_2$ in various concentrations. In this experiment, spleen cells were used to assay many samples at the same time under the same conditions. As shown in Table 1, maximum IgE PFC were induced by PWM 10 μl/ml plus $HgCl_2$ 1×10^{-7}M, whereas neither IgG PFC (nor IgM or IgA PFC, results not shown) were not significantly different with PWM 10 μl/ml plus $HgCl_2$ 1×10^{-7}M, 1×10^{-6}M or no $HgCl_2$ respectively. Less PFC were produced in every class when cultured with PWM 0·1 μl/ml or 1 μl/ml either alone or with various concentrations of $HgCl_2$.

Further investigations were carried on peripheral MNC. Unfractionated MNC were cultured with PWM 10 μl/ml plus various concentrations of $HgCl_2$. The addition of $HgCl_2$ to PWM had no

Table 1. Synergy of PWM and $HgCl_2$ in induction of ISC

	PWM (μl/ml)					
	IgG PFC/2 × 10^4 cells			IgE PFC/2 × 10^5 cells		
$HgCl_2$ (M)	0·1	1	10	0·1	1	10
0	26±6	84±1	381±31	3±1	5±1	9±9
1×10^{-7}	14±8	89±6	473±122	1±0	4±2	40±1
1×10^{-6}	13±3	18±8	375±24	4±1	1±1	11±0
1×10^{-5}	1±1	2±2	1±1	0±0	0±0	1±0
$3{\cdot}5 \times 10^{-5}$	1±1	1±0	0±0	0±0	0±0	0±0

Spleen cells were cultured with various concentrations of PWM plus $HgCl_2$. This patient with hereditary spherocytosis had normal serum IgG, IgM, IgA and IgE. Peak PFC were detected on day 5.

effect on IgG PFC (nor IgM or IgA PFC, result not shown), while cultures with PWM plus $HgCl_2$ 5×10^{-8}M induced significantly more IgE PFC than other cultures (Fig. 2). The same investigations were performed with MNC from a patient with asthma. As shown in Fig. 3, the addition of $HgCl_2$ 5×10^{-8}M to PWM 10 μl/ml induced a selective IgE PFC increase without affecting IgG, IgM and IgA PFC production. This was confirmed with cells from other normal donors and patients with

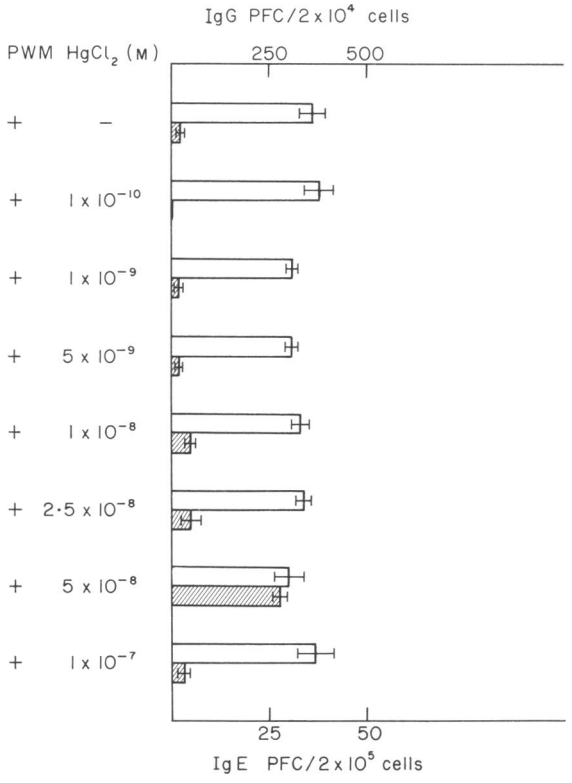

Fig. 2. Synergy of PWM and $HgCl_2$ in induction of ISC in cells of normal donors. MNC of normal donors were cultured with PWM 10 μl/ml or PWM 10 μl/ml plus various concentrations of $HgCl_2$. Peak PFC were detected on day 7. Only IgG (□) and IgE (■) PFC are shown.

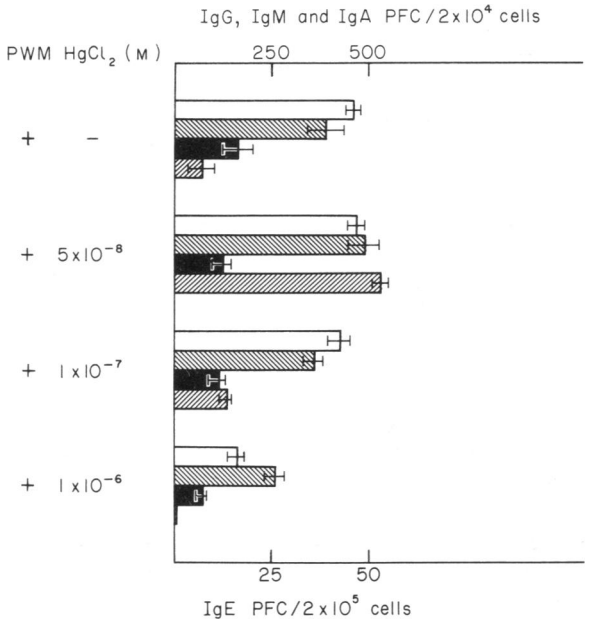

Fig. 3. Synergy of PWM and $HgCl_2$ in induction of ISC in cells of asthma patient. MNC of a patient with asthma were cultured with PWM 10 μl/ml plus various concentrations of $HgCl_2$. Peak PFC of IgG (□), IgM (■), IgA (■) and IgE (■) were detected on day 6.

Table 2. Synergy of PWM and $HgCl_2$ in cells from 16 normal donors

	IgE PFC/2×10^5 cells	
Donor	PWM	PWM + $HgCl_2$
1 (6)*	1±1	43±13
2 (7)	15±2	9±1
3 (7)	5±3	32±0
4 (6)	24±2	39±6
5 (7)	20±3	55±13
6 (7)	15±1	28±12
7 (6)	5±2	38±28
8 (7)	10±2	38±22
9 (7)	1±1	28±2
10 (7)	50±14	44±10
11 (6)	2±1	25±5
12 (6)	0±0	5±2
13 (7)	0±0	26±0
14 (6)	32±12	93±2
15 (6)	59±9	108±21
16 (7)	0±0	0±0

Unfractionated MNC were cultured with PWM 10 μl/ml or PWM 10 μl/ml plus $HgCl_2$ 5×10^{-8}M. Peak PFC were detected in each donor.

* In parenthesis, day of maximum response in each culture.

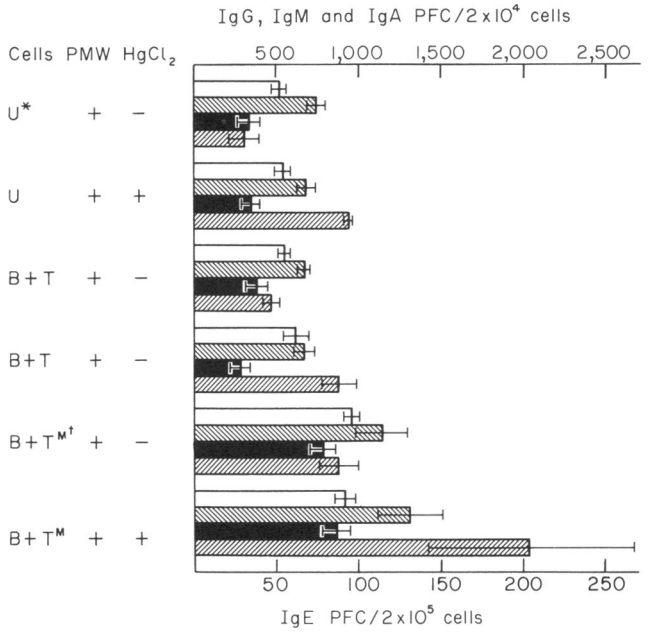

Fig. 4. Effect of MMC treated T cells in induction of ISC. MNC from normal donor were cultured with PWM 10 μl/ml or 10 μl/ml plus $HgCl_2$ 5×10^{-8}M as unfractionated (*), B cells plus T cells, B cells plus MMC treated T cells (+). Peak PFC of IgG (□), IgM (▧), IgA (■) and IgE (■) were detected on day 6.

Table 3. Effect of MMC treated T cells

	IgE PFC/2×10^5 cells			
	PWM		PWM + $HgCl_2$	
donor	B+T	B+T^{M*}	B+T	B+T^M
1 (7)†	ND‡	12±1	ND	153±72
2 (5)	10±2	13±7	14±4	22±0
3 (6)	46±1	88±12	83±21	225±63
4 (5)	36±1	97±7	51±3	134±2

Combinations of B cells plus T cells or B cells plus MMC treated T cells from normal donors were cultured with PWM 10 μl/ml or PWM 10 μl/ml plus $HgCl_2$ 5×10^{-8}M, and peak IgE PFC were detected. Though not shown, IgG, IgM and IgA PFC were not significantly different in each culture.

* MMC treated T cells.

† In parenthesis, day of maximum response in each culture.

‡ ND = not done.

high serum IgE. Some patients produced many IgE PFC even with PWM alone, while others did not (not shown). We, therefore, used PWM 10 μl/ml and $HgCl_2$ 5×10^{-8}M in subsequent cultures. IgE PFC were detected in cells from 16 normal donors. However, we found various responses. As shown in Table 2, in 13 of the 16 donors IgE increased in various degrees (donors 1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14 & 15), while in one it was unchanged (donor 10), in one it decreased (donor 2) and in one IgE PFC were not induced (donor 16). Table 2 also shows that when PWM alone induced IgE PFC, PWM plus $HgCl_2$ increased it even more and selectively, while the addition of $HgCl_2$ to PWM did not affect IgG, IgM or IgA PFC. There was no correlation between IgE and the other classes of PFC (not shown).

MMC treated T cells increased IgE PFC when cultured with autologous B cells

MNC of normal donors were divided into the following groups: unfractionated, B cells plus T cells, B cells plus MMC treated T cells. In each group (U, B + T, B + T^M), the addition of $HgCl_2$ produced more IgE PFC without affecting other classes of PFC. In co-cultures of B cells plus MMC treated T cells, PWM alone produced many IgE PFC, however, more IgE PFC were generated by PWM plus $HgCl_2$ (Fig. 4). IgE PFC from four different donors showed various responses; in three IgE PFC were significantly increased by PWM plus $HgCl_2$, while in one the increase was only slight (Table 3).

DISCUSSION

These results demonstrate that a combination of PWM and $HgCl_2$ increases IgE PFC selectively, although PWM alone produces less IgE PFC, and $HgCl_2$ alone induces no significant PFC.

PWM-induced Ig production in human B lymphocytes depends on T lymphocytes (Janossy & Greaves, 1975). Saiki & Ralph (1981) reported that low concentrations of PWM plus Cowan I had additional effects on ISC induction. In the meantime, $HgCl_2$ has been shown to exert mitogenic activity on whole human lymphocyte populations (Carson, Poutala & Prouvost, 1979), enhancement of β_2-microglobulin formation (Ohsawa & Kimura, 1979) and leucocyte aggregation (Hutchinson, Macleod & Raffle, 1976). Prouvost-Danon *et al.* (1981) showed in Brown-Norway rats, but not in Lewis rats, that $HgCl_2$ potentiated not only total IgE response but also a specific anti-ovalbumin IgE response when the rats were immunized with ovalbumin. Brown–Norway and Lewis rats are high and low IgE responders respectively (Bennich, Ellerson & Karlsson, 1978; Abadie & Prouvost-Danon, 1980). The final concentration of $HgCl_2$ used in this study differed from theirs. However, the optimal conditions for each mitogen appear to change according to the method of application; i.e., whether each is used separately or two mitogens are used at the same time, as with the synergy of Cowan I and PWM (Saiki & Ralph, 1981).

There are two main questions to be solved: (1) the mechanism of selective IgE increase by synergy of PWM and $HgCl_2$ and (2) the reason for the various responses among donors, including the absence of IgE PFC increase in the cells from some donors. In the first place, we found that PWM plus $HgCl_2$ increased IgE PFC more in cases in which PWM alone induced many IgE PFC. This indicates that $HgCl_2$ potentiates PWM-induced IgE responses in one or more of the following ways: (a) it may inhibit IgE specific suppressor T cells, (b) it may affect directly IgE B cells, (c) it may affect certain monocytes, or (d) it may promote IgE specific helper T cells, in PWM stimulated cultures. The inhibition of IgE specific T cell suppression is unlikely for the following reasons. With stimulation by PWM alone, co-cultures of B cells plus MMC treated T cells produced more IgE PFC than did B cells plus T cells. However, large numbers of IgE PFC were generated in co-cultures of B cells plus MMC treated T cells when stimulated by PWM plus $HgCl_2$. Nevertheless, the possibility of the existence of MMC resistant IgE specific suppressor T cells which are $HgCl_2$ sensitive can not be excluded.

The effect on IgE B cells or monocytes remains obscure. Ohsawa & Kimura (1979) suggested that mercuric ion causes a proliferative response of lymphocytes by a mechanism different from that for the stimulation by phytohaemagglutinin (PHA). It is possible that $HgCl_2$ stimulates IgE B cells or some monocytes which are not stimulated by PWM, though $HgCl_2$ alone failed to induce any ISC. We have no clear data yet to support these hypotheses.

The promotion of IgE specific T cell help is suggested by the striking increase of IgE PFC when B cells plus MMC treated T cells are stimulated by PWM plus $HgCl_2$. In non-atopic people, cells which produce IgE in PWM driven cultures, are surface membrane (Sm) IgE^+, IgM^+ B lymphocytes which require T cell help (Saxon *et al.*, 1980b). On the contrary, in atopic patients, cells which produce IgE spontaneously *in vitro*, are $SmIgE^+$, IgM^-, DR^+, CR^- circulating lymphocytes which do not require T cell help (Romagnani *et al.*, 1980a, 1980b, 1982a). The addition of autologous T cells to those B cells produced less IgE when cultured with or without PWM than did B cells alone spontaneously (Fiser & Buckley, 1979; Saxon *et al.*, 1980a). The former may be activated and transformed into the latter *in vitro* in atopic patients. In neonatally thymectomized rats, the development of IgE bearing cells occurred, but enhancement of IgE synthesis after infection with *Nippostrongylus brasiliensis* (Nb) did not occur (Urban, Ishizaka & Ishizaka, 1979). In our culture system also, IgE production is highly T cell-dependent. It is likely that $HgCl_2$ activates IgE specific helper T cells which are not activated by PWM as $HgCl_2$ *vs* PHA(Ohsawa & Kimura, 1979).

Yodoi & Ishizaka (1979) described a transition of $Fc\gamma R(+)$ cells to $Fc\varepsilon R(+)$ cells caused by IgE. Suemura & Ishizaka (1979) reported that T cells from rats infected with Nb released soluble factors that specifically enhanced IgE response. Similar mechanisms are also considered to operate in this effect of $HgCl_2$. Nevertheless, definite details await further investigation.

Second, unlike rats, heterogeneous human populations do not all produce ISC in response to PWM (Nagel, Chrest & Adler, 1981). Thus, it is not unlikely that there are subpopulations of high and low responders to PWM and $HgCl_2$ in terms of IgE production. Though none of our series had a history of sensitivity to mercury and in all of them PWM produced enough IgG, IgM and IgA PFC, each donor might show various responses depending on his or her own subpopulation. As a result, in some IgE PFC increased, and in others it did not. Moreover, the optimal concentrations of $HgCl_2$ or PWM may vary for each donor. Zuraw *et al.* (1981) reported that in hyper-IgE patients when the PWM dose was reduced to 0·1 μg/ml, it was possible to increase IgE production above the unstimulated base line value. The difference in IgE production with PWM alone and PWM plus $HgCl_2$ seems to reflect various optimal concentrations of each mitogen when used together in cells from different donors.

Experiments are in progress to elucidate the mechanisms involved. The *in vitro* potentiation of the IgE production system without affecting other Ig should be very useful.

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