Clin. exp. Immunol. (1992) **87**, 53–57

Hyper-response of serum IgG1 to *Staphylococcus aureus* peptidoglycan in patients with hyper-IgE syndrome

A. ISHIZAKA, K. KOJIMA, Y. SAKIYAMA, S. MATSUMOTO, K. KUWAJIMA, Y. WAGATSUMA, R. SHIBATA* & K. JOH† *Department of Pediatrics, Hokkaido University School of Medicine, Sapporo, *Minami Fukuoka National Hospital, Fukuoka, and †Saitama Children's Medical Center, Saitama, Japan*

(Accepted for publication 2 July 1991)

SUMMARY

The hyper-IgE (HIE) syndrome is characterized by high IgE serum levels, chronic dermatitis and recurrent infections. To determine whether an impairment of the antibody response to *Staphylococcus aureus* contributes to infections in this syndrome we measured total serum IgG subclass, specific IgG1 and IgG2 levels against peptidoglycan (PG), the immunodominant cell wall component of *S. aureus* and serum opsonic activity to PG. Of the 14 patients with HIE syndrome, nine had increased level of serum IgG1 and six had IgG2 subclass deficiency. In regard to specific response of IgG1 and IgG2 antibodies to PG, patients were divided into five groups related to ages and compared with 10 control subjects for each age cohort. Patients with HIE syndrome had significant high levels of serum-specific IgG1 to PG and significant decreased levels of serum-specific IgG2 to PG in all five groups. Additionally, serum opsonic activity in patients was significantly higher than that in normal control subjects. It is concluded that IgG2 deficiency or poor IgG2 antibody response to *S. aureus* is not the explanation of the abnormal susceptibility to *S. aureus* infections of HIE patients.

Keywords hyper IgE syndrome IgG subclass *Staphylococcus aureus* peptidoglycan opsonic activity

INTRODUCTION

The hyper-IgE (HIE) syndrome is a rare disorder characterized by markedly elevated serum IgE, chronic dermatitis, and serious recurrent infections [1–3]. Although a neutrophil chemotactic defect may be found in patients, the basis of the undue susceptibility to infections is as yet poorly understood [4–6]. Leung *et al.* [7] recently reported an impaired antibody response to teichoic acid (TA), surface polysaccharide of *Staphylococcus aureus*, associated with IgG2 deficiency in four of seven patients studied. On the other hand, Aucouturier *et al.* [8] failed to confirm the data of Leung *et al.* [7], showing that IgG subclass levels were normal in five of six patients.

Of staphylococcal products for increasing adherence or interfering with the host defence mechanism, peptidoglycan (PG), the immunodominant cell wall component of *S. aureus*, seems to be more important than TA, because PG and not TA can induce luminol-dependent chemiluminescence (CL) of polymorphonuclear leucocytes (PMNL) in the presence of specific antibody to relevant antigen [9]. In the present study, patients with HIE syndrome were evaluated for total IgG subclass levels and for IgG1 and IgG2 levels to PG. Furthermore, serum opsonic activity to PG was also studied. Our results indicate that six of 14 patients had IgG2 subclass deficiency and most patients had an impaired IgG2 response to PG. In contrast, patients had an increased level of serum IgG1 as well as an increased IgG1 response to PG. In spite of the imbalance of IgG subclass response, an increased opsonic activity to PG was demonstrated in patients with HIE compared with age-matched control subjects. It is concluded that IgG2 deficiency or poor IgG2 antibody response to *S. aureus* is not the explanation of the abnormal susceptibility to staphylococcal infections of HIE patients.

Correspondence: A. Ishizaka, Department of Pediatrics, Hokkaido University School of Medicine, N15 W7, Kita-ku, Sapporo, 060, Japan.

PATIENTS AND METHODS

Study population

Fourteen patients with HIE syndrome (mean age 9·5 years; range 6 months to 30 years) were studied. The diagnosis of HIE syndrome was based on a history of recurrent sinusitis, pneumonia, deep-seated skin and pulmonary abscesses predominantly caused by *S. aureus*, chronic eczematoid dermatitis, and marked elevation of serum IgE (mean IgE, 15 700 U/ml; range, 4000–41 000 U/ml). None of the patients received replacement therapy with i.v. immunoglobulin. Fifty healthy subjects were studied for anti-PG-specific IgG1 and IgG2 as control for each age cohort.

IgG subclass determination

Serum IgG subclass levels were evaluated by standard ELISA, using monoclonal antibodies (MoAb) against human IgG subclass (HP6069 for IgG1, HP6014 for IgG2, HP6050 for IgG3, and HP6011 for IgG4; Oxoid Ltd, Hampshire, UK) [10]. WHO-REF-SERA 67/97 was used as a reference.

Assay for serum anti-PG IgG1 or IgG2 antibody levels

The assay for antibody to PG was done by the method of Kojima *et al.* [9]. In brief, PG was prepared from *S. aureus* strain FDA209P JC-1 by the method of Peterson *et al.* [11] and was used at a concentration of 10 μg/ml to coat microtitre plates. After blocking non-specific sites with 0·2 ml of 0·25% gelatin in phosphate-buffered saline (PBS), 80 μl of 10-fold diluted serum were incubated for 1·5 h at room temperature. Then, after washing four times with PBS-Tween, 80 μl of mouse MoAbs, HP6069 for IgG1 or HP6014 in combination with HP6002 (Oxoid) for IgG2, were added and incubated for 1 h at room temperature, followed by the addition of a 1/2000 dilution of peroxidase-conjugated, goat anti-mouse IgG (Tago Inc., Burlingame, CA) for 1 h at room temperature. After washing four times with PBS-Tween, 80 μl of *o*-phenylenediamine (34 mg/dl; Kanto Chemical Co., Tokyo, Japan) plus hydrogen peroxide (15 μl/dl, 30% v/v solution) in citrate-phosphate buffer (pH 5·0, 0·15 M) were added. The reaction was stopped by adding 50 μl of 12·5% sulphuric acid and the plates were read on a VMAX (Molecular Devices Corp., Palo Alto, CA) at 492 nm. Relative IgG1 and IgG2 levels to PG were based on A_{492} for each serum sample after subtracting the A_{492} of the background wells (always $<0·05$).

Assay for opsonic activity to PG

Opsonic activity of sera obtained from patients was determined by measuring their chemiluminescence values. In brief, PG (10 μg/ml) was opsonized with 100 μl of serum at 37°C for 30 min with 100 μl of fetal calf serum (FCS) as a source of complement. PMNL were isolated by the methylcellulose method from heparinized venous blood from healthy adult donors and adjusted to a concentration of 5×10^5/ml in Hank's balanced salt solution. CL was initiated by the addition of opsonized PG into liquid scintillation vials (Wheaton Scientific, Millville, NJ) containing 0·5 ml of PMNL suspension and 15 μl of luminol (2 mg/ml; Sigma Chemical Co., St Louis, MO). After incubation with stirring for 20 min at room temperature, the CL value was counted by the Luminescence Reader Model BLR-102 (Aloka, Tokyo, Japan) for 6 s. All samples were measured in triplicate and averaged.

Statistical analysis

Significant differences in serum anti-PG IgG1 and IgG2 levels or an opsonic activity to PG were determined in a paired Student's *t*-test.

RESULTS

Serum IgG subclass level in 14 patients with HIE syndrome was determined by ELISA (Fig. 1 and Table 1). Serum IgG1 levels were increased in nine of 14 patients. On the other hand, six of 14 patients had IgG2 deficiency defined as a serum IgG2 subclass level >2 s.d. below the mean for their age (serum IgG2 levels were as follows; 29, 31, 46, 51, 52 and 61 mg/dl). Three patients showed IgG3 deficiency (IgG3 levels: 3, 5 and 15 mg/dl). An increased level of serum IgG4 was remarkably pronounced in patients with an increased level of serum IgE. A high correlation was seen between serum IgE and IgG4 levels ($r=0·75$, $P<0·005$). In contrast, other IgG subclass levels were not remarkably correlated with serum IgE level ($r=0·21$, 0·43 and 0·41 in IgG1, IgG2 and IgG3, respectively).

Next, IgG1 and IgG2 levels to PG were determined in 14 patients with HIE syndrome. Patients were divided into five groups related to their ages and studied in parallel with 10 healthy controls for each age cohort. As shown in Fig. 2a, in healthy controls there was no age-related variation in anti-PG-specific IgG1 levels. The patients with HIE syndrome had significantly higher levels of anti-PG IgG1 than age-related normal control healthy subjects except the <3 years group (P values were 0·0238, 0·0070 and 0·0322 for 6–10 years, 11–13 years and adult group, respectively, and 0·2383 for the <3 years group). In contrast, there were age-related variations between control subjects in anti-PG IgG2 levels (Fig. 2b). Namely, the anti-PG IgG2 level in the <3 years group of healthy subjects was significantly decreased compared with those in the 11–13 years and adult control group ($P<0·01$ and 0·001, respectively). Anti-PG IgG2 levels in the 3–5 years and 6–10 years control groups were significantly decreased compared with the adult control group ($P<0·01$ and $<0·05$, respectively). Compared with age-related control healthy subjects, patients with HIE syndrome had significantly lower levels of anti-PG IgG2 in all age-related groups (P values were 0·0400, 0·0151, 0·0017 and 0·0001 for <3 years, 6–10 years, 11–13 years and the adult group, respectively). These results showed that patients with HIE syndrome had the imbalance of IgG subclass production, with regard not only to total amount of serum IgG subclass but also to anti-PG-specific IgG subclass response.

To determine whether the imbalance of serum IgG subclass response to PG had influences on opsonic activity against *S. aureus*, serum opsonic activity to PG in patients with HIE syndrome was measured by the method of chemiluminescence and compared with that in normal control subjects. As shown in Table 2, serum opsonic activity in patients with HIE syndrome was significantly higher than that in normal control subjects ($P<0·005$). These results suggested that patients with HIE syndrome had an adequate opsonic activity to PG in spite of decreased levels of serum IgG2 and anti-PG-specific IgG2.

DISCUSSION

Defence against bacterial infections is mediated by antibody, complement and phagocytes. There is no evidence in HIE syndrome of an abnormality in the complement system, and extensive investigations of neutrophil function in this syndrome reveal normal capacity to phagocytize and kill bacteria [3]. Although the neutrophil chemotactic defect is also reported in this syndrome, it is believed that this defect is a secondary rather than a primary immunologic abnormality.

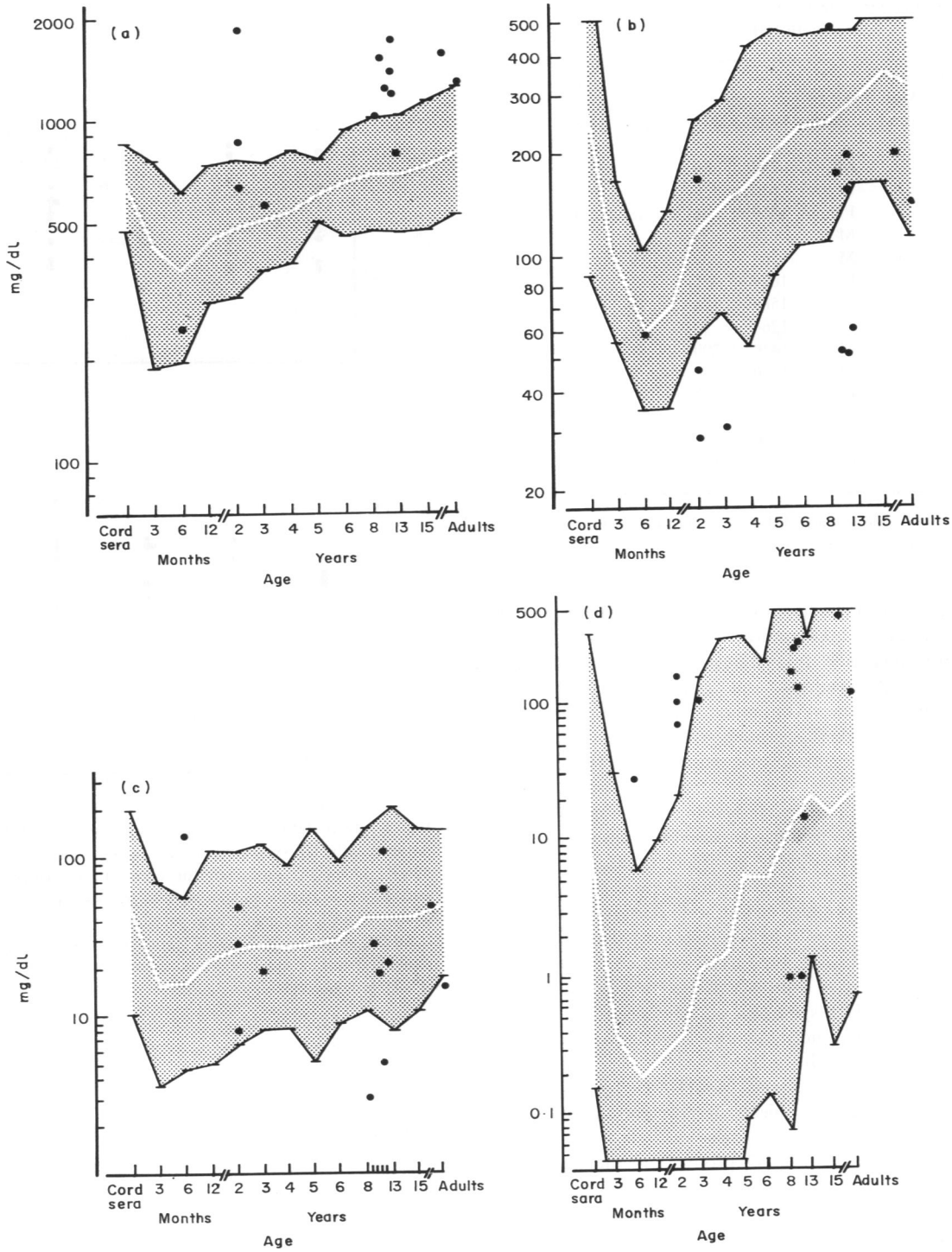

Fig. 1. Serum concentrations of IgG1 (a), IgG2 (b), IgG3 (c) and IgG4 (d) in patients with HIE syndrome. ●, Patients. Shaded area denotes mean ±2 s.d. interval for age.

Several previous observations suggest that patients with HIE syndrome have impairment of their humoral immune response: (i) low baseline diphtheria and tetanus toxoid antibody titre and decreased anamnestic antibody response to DPT or DT booster immunizations [12]; (ii) a deficiency of serum IgA antibody against whole *S. aureus* and the absence of the expected excess of anti-*S. aureus* IgG despite an excess of anti-*S. aureus* IgM [13]; (iii) low levels of serum antibody to the carbohydrate antigens, *Haemophilus influenzae* type b and TA, and an absolute IgG2 subclass deficiency [7].

The recent availability of assays to measure accurately human IgG subclasses enables us to investigate not only serum IgG subclass levels but also antigen-specific IgG subclass levels. In this study serum IgG subclass levels in patients with HIE syndrome were measured using an ELISA with MoAbs because there was a conflict with regard to serum IgG subclass levels in

Table 1. Serum concentrations of IgG subclasses and IgE in patients with HIE syndrome

Patient no.	Age (years)	Sex	mg/dl IgG1	IgG2	IgG3	IgG4	IgE (U/ml)
1	1/2	M	247	60	136	28	9000
2	2	M	630	170	28	107	15400
3	2	F	1850	46	48	160	12000
4	2	M	870	29	8	72	12000
5	3	M	562	31	19	107	11000
6	8	F	1024	485	3	1	12000
7	9	F	1560	176	28	176	13000
8	10	F	1264	52	18	256	20800
9	11	M	1410	200	61	130	17000
10	11	M	1760	51	5	1	4000
11	11	F	1216	157	107	285	26000
12	12	F	800	61	21	15	9100
13	20	M	1600	204	42	450	41000
14	30	F	1306	145	15	122	18000

Serum IgG subclass and IgE levels were determined by standard ELISA and radioimmunoassay, respectively.

this syndrome. Our results show that six of 14 patients had IgG2 subclass deficiency and confirm the data of Leung *et al.* [7]. Next, antigen-specific IgG subclass levels were studied in patients with HIE syndrome and age-matched healthy control subjects. There are several immunodominant antigens of *S. aureus* reported, e.g. TA, PG, protein A, leukocidin, *et al.* [14]. Of these antigens it is reported that patients with HIE syndrome lacked the expected elevation of serum antibody to TA [7]. However, in our preliminary study using the chemiluminescence method, TA failed to produce superoxide from neutrophils in the presence of antibody and complement. Considering that TA, although it is a major immunodominant surface antigen of *S. aureus*, does not produce an antibody response with opsonic activity, PG was chosen to be studied rather than TA because PG provoked an adequate superoxide production from neutrophils in the presence of antibody and complement. We found that patients with HIE syndrome had significantly lower levels of anti-PG IgG2 than normal control healthy subjects. Unexpectedly, compared with the anti-PG IgG2 level, the patients had significantly higher levels of anti-PG IgG1 level than normal control healthy subjects. It is hypothesized that this excess of anti-PG IgG1 production seems to be a compensation for a defect of anti-PG IgG2 production and provides an adequate humoral immune defense against *S. aureus*. In order to demonstrate this hypothesis, serum opsonic activity to PG of patients with HIE syndrome was measured by the chemiluminescence method in the presence of complement. Our results showed that patients with HIE syndrome had an adequate opsonic activity to PG in spite of decreased levels of serum IgG2 and anti-PG-specific IgG2. We therefore conclude that the impaired PG-specific IgG2 responses and IgG2 deficiency do not contribute to the increased susceptibility to *S. aureus* infections in the HIE syndrome.

Recently, it has been demonstrated that human recombinant IL-4 induces the production of IgE by peripheral blood lymphocytes and that IL4-induced IgE production is blocked by interferon-gamma (IFN-γ) [15,16]. Thus, imbalances between

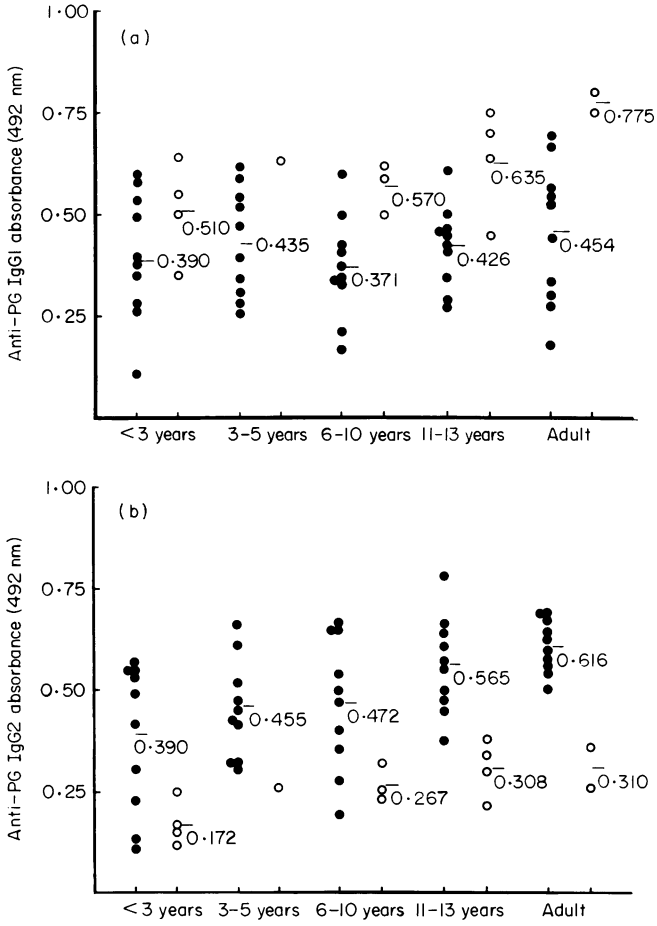

Fig. 2. Serum IgG1 (a) and IgG2 (b) levels of anti-PG antibody in patients with HIE syndrome (○) and age-matched control subjects (●). Mean values are presented for each group. Serum anti-PG IgG1 antibody was significantly higher in patients with HIE syndrome as compared with normal control subjects except the < 3 years group. In contrast, serum anti-PG IgG2 antibody was significantly lower in patients with HIE syndrome as compared with normal control subjects in all age-related groups.

Table 2. Serum opsonic activity to PG by chemiluminescence

Group (*n*)	CL value (ct/min, mean ± s.d.)
Patients with HIE syndrome (5)	37330 ± 3640
Healthy donors (10)	16570 ± 5200

Opsonized PG was prepared by incubation of PG (10 μg/ml) with 100 μl of serum obtained from patients or healthy donors and 100 μl of FCS at 37°C for 30 min. CL was initiated by the addition of opsonized PG into liquid scintillation vials containing 0·5 ml of PMNL suspension and 15 μl of luminol (2 mg/ml). After incubation with stirring for 20 min at room temperature, the CL value was counted by the Luminescence Reader for 6 s. All samples were measured in triplicate and averaged. Serum opsonic activity in patients with HIE syndrome was significantly higher than that in normal control subjects ($P < 0{\cdot}005$).

IL4-producing and IFN-γ-producing helper T cells seem to be responsible for polyclonal IgE production in patients with the HIE syndrome [17]. At present it is controversial whether there is a significant difference of cytokine production by peripheral blood mononuclear cells between HIE patients and normal controls [18–20]. However, it has been observed that the number of CD23/Fcε RIIb$^+$ cells has been found to be increased in patients with HIE syndrome. This indirectly suggests that IL-4 may be abnormally secreted in HIE patients, because the expression of Fcε RIIb on B cells is IL-4-dependent. There are few studies about the regulation of IgG subclass production by cytokines [21,22]. King *et al.* [18] reported that *in vivo* IFN-γ treatment affected serum IgG subclass production with changes in serum IgE in patients with this syndrome. In our *in vitro* study, recombinant IL-4 enhanced not only spontaneous IgE synthesis but also IgG4 synthesis in cultures of peripheral blood lymphocytes from patients with HIE syndrome as well as from healthy donors, and the effect of recombinant IL-4 on both IgE and IgG4 synthesis was inhibited by low concentrations of recombinant IFN-γ [23]. Thus, the imbalance of serum IgG subclasses may also be derived from an imbalance in cytokine production (i.e. increased release of IL-4 and/or decreased release of IFN-γ). Recently, it has been demonstrated that IL-4 suppresses both IFN-γ production at protein and mRNA level and tumour necrosis factor-α (TNF-α) [24,25]. Furthermore, to our interest, the recent data based on the *in vitro* and *in vivo* studies demonstrated that IFN-γ and TNF-α are able to enhance the neutrophil oxidative burst in response to a second stimulus [26,27]. It is tempting to speculate that the disturbed regulation of cytokines by patients' T cells play an important role in preventing the activation of phagocytic cells at a local level, thus escaping our detection.

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Health and Welfare, Japan, the Mother and Child's Health Foundation, Japan and the Tokyo Biochemical Research Foundation, Japan.

REFERENCES

1 Buckley RH, Sampson HA. The hyperimmunoglobulinemia E syndrome. In: Franklin EC ed. Clinical Immunology Update. Elsevier/North Holland Biomedical Press, New York, 1981:148.
2 Rosen FS, Wedgwood RJ, Eibl M. Primary immunodeficiency diseases. Report of a World Health Organization scientific group. Clin Immunol Immunopathol 1986; **9**:722.
3 Geha RS, Leung DYM. Hyper immunoglobulin E syndrome. Immunodef. Rev. 1989; **1**:155.
4 Hill HR, Quie PG. Raised serum-IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. Lancet 1974; **i**:183.
5 Hill HR, Quie PG, Pabst HF, Ochs HD, Clark RA, Klebanoff SJ, Wedgwood RJ. Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. Lancet 1974; **ii**:617.
6 Donabedian H, Gallin JI. Mononuclear cells from patients with the hyperimmunoglobulin E recurrent-infection syndrome produce an inhibitor of leukocyte chemotaxis. J Clin Invest 1982; **69**:1155.
7 Leung DYM, Ambrosino DM, Arbeit RD, Newton JL, Geha RS. Impaired antibody responses in the hyperimmunoglobulin E syndrome. J Allergy Clin Immunol 1988; **81**:1082.
8 Aucouturier P, Lacombe C, Bremard-Oury C, Lebranchu Y, Griscelli C, Preud'homme J-L. Normal IgG subclass levels in the hyperIgE syndrome. Immunol Lett 1989; **21**:329.
9 Kojima K, Ishizaka A, Taguchi Y *et al.* Opsonic activity of intravenous immunoglobulin preparations for the cell wall components of *Staphylococcus aureus*. Vox Sang, 1991; in press.
10 Ishizaka A, Nakanishi M, Yamada S, Sakiyama Y, Matsumoto S. Development of hypogammaglobulinaemia in a patient with common variable immunodeficiency. Eur J Pediatr 1989; **149**:175.
11 Peterson PK, Wilkinson BJ, Kim Y, Schemeling D, Douglas ST, Quie PG, Verhoef J. The key role of peptidoglycan in the opsonization of *Staphylococcus aureus*. J Clin Invest. 1978; **61**:597.
12 Buckley RH, Becker WG. Abnormalities in the regulation of human IgE synthesis. Immunol Rev 1978; **41**:288.
13 Dreskin SC, Goldsmith PK, Gallin JI. Immunoglobulins in the hyperimmunoglobulin E and recurrent infection (Job's) syndrome. Deficiency of anti-*Staphylococcus aureus* immunoglobulin A. J Clin Invest 1985; **75**:26.
14 Cohen ML. *Staphylococcus aureus:* biology, mechanisms of virulence, epidemiology. J Pediatr 1986; **108**:796.
15 Pene J, Rousset F, Briere F *et al.* IgE production by normal human lymphocytes is induced by IL-4 and suppressed by interferon γ and α and prostaglandin E_2. Proc Natl Acad Sci USA 1988; **85**:6880.
16 Yang XD, De Weck AL, Stadler BM. Effect of recombinant human interleukin 4 on spontaneous *in vitro* human IgE synthesis. Eur J Immunol 1988; **88**:1699.
17 Prete DG, Tiri A, Maggi E *et al.* Defective *in vitro* production of γ-interferon and tumor necrosis factor-α by circulating T cells from patients with the hyper-immunoglobulin E syndrome. J Clin Invest 1989; **84**:1830.
18 King CL, Gallin JI, Malech HL, Abramson SL, Nutman TB. Regulation of immunoglobulin production in hyperimmunoglobulin E recurrent-infection syndrome by interferon γ. Proc Natl Acad Sci USA 1989; **86**:10085.
19 Paganelli R, Scala E, Capobianchi MR *et al.* Selective deficiency of interferon-gamma production in the hyper-IgE syndrome. Relationship to *in vitro* IgE synthesis. Clin Exp Immunol 1991; **84**:28.
20 Vercelli D, Jabara HH, Cunningham-Rundles C *et al.* Regulation of immunoglobulin (Ig) E synthesis in the hyper-IgE syndrome. J Clin Invest 1990; **85**:1666.
21 Ishizaka A, Sakiyama Y, Nakanishi M *et al.* The inductive effect of interleukin-4 on IgG4 and IgE synthesis in human peripheral lymphocytes. Clin Exp Immunol 1990; **79**:392.
22 Lundgren M, Persson U, Larsson P *et al.* Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. Eur J Immunol 1989; **19**:1311.
23 Ishizaka A, Joh K, Shibata R *et al.* Regulation of IgE and IgG4 synthesis in patients with hyper IgE syndrome. Immunology 1990; **70**:414.
24 Peleman R, Wu J, Fargeas C, Delespesse G. Recombinant Interleukin 4 suppresses the production of interferon γ by human mononuclear cells. J Exp Med 1989; **170**:1751.
25 Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN-γ and induces the synthesis of IgE in human mixed lymphocyte cultures. J Immunol 1990; **144**:570.
26 Berkow RL, Wang D, Larrick JW, Dodson RW, Howard TH. Enhancement of neutrophil superoxide production by preincubation with recombinant human tumor necrosis factor. J Immunol 1987; **139**:3783.
27 Te Velde AA, Huijbens RJF, Heije K, De Vries JE, Figdor CG. Interleukin-4 (IL-4) inhibits secretion of IL-1, tumor necrosis factor α, and IL-6 by human monocytes. Blood 1990; **76**:1392.