cerebral lactate accumulation occur with seizures,[25] but this does not persist unless the seizures are repetitive or continuous or cerebral oxygen supply is reduced. Lumbar puncture was not performed within 6 h of a convulsion in this series, so it is unlikely that seizures were a major contribution to CSF lactate accumulation.

The most likely explanation for the elevation of CSF lactate and the inverse correlation between CSF lactate and glucose in this study is that there is cerebral hypoxia in cerebral malaria, probably related to sequestration of parasitised red cells in the cerebral microcirculation, and this leads to anaerobic glycosis in the brain. Persisting neurological deficit is not a feature of cerebral malaria, which suggests that in survivors, at least, the brain is protected from excessive intracellular acidosis. In the mouse, fulminant *P yeolii* infections are not associated with a fall in cerebral pH despite severe acidaemia.[26] Local or systemic hypoglycaemia may be protective in this respect.[27] CSF lactate reflected the severity of cerebral malaria and predicted the prognosis with greater precision than any other single clinical sign or laboratory result. All patients with a CSF lactate concentration over 6 mmol/l died. This measurement may therefore be of value in the assessment and planning of treatment of patients with cerebral malaria.

We thank the director of Pra Pokklao hospital, Dr Chaisit Dharakul, and his staff for their help, Mrs Kamolrat Silamut and Mrs Vanaporn Wutiekanun for excellent technical help, Dr Sanjeev Krishna for his advice, and Miss Eunice Berry for preparing the manuscript. This study was part of the Wellcome-Mahidol University, Oxford Tropical Medicine research programme funded by the Wellcome Trust.

Correspondence should be addressed to D. A. W., Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Rd, Bangkok 10400, Thailand.

REFERENCES

1. Warrell DA, Looareesuwan S, Warrell MJ, Intaraprasert R, Kasemsarn B, Bunnag D, Harinasuta T. Dexamethasone proves deleterious in cerebral malaria: Double blind trial in 100 comatose patients. *N Engl J Med* 1982; **306:** 313–19.
2. Looareesuwan S, Warrell DA, White NJ, et al. Do patients with cerebral malaria have cerebral oedema? *Lancet* 1983; i: 434–47.
3. Miller LH, Usami S, Chien S. Alterations in the rheologic properties of *Plasmodium knowlesi* infected red cells. A possible mechanism for capillary obstruction. *J Clin Invest* 1971; **50:** 1451–55.
4. Udeinya IJ, Schmidt JA, Aikawa M, Miller LH, Green I. Falciparum malaria infected erythrocytes specifically bind to cultured human endothelial cells. *Science* 1981; **213:** 555–57.
5. White NJ, Warrell DA, Looareesuwan S, Chanthavanich P, Bunnag D, Harinasuta T. Quinine loading dose in cerebral malaria. *Am J Trop Med Hyg* 1983; **32:** 1–5.
6. Fishman RA. Cerebrospinal fluid in diseases of the nervous system. Philadelphia: WB Saunders, 1980.
7. Siesjö B. Brain energy metabolism. Chichester: John Wiley, 1978.
8. Pardridge WM, Oldenorf WH. Transport of metabolic substrates through the blood-brain barrier. *J Neurochem* 1977; **28:** 512–.
9. Posner JB, Plum F. Independence of blood and cerebrospinal fluid lactate. *Arch Neurol* 1967; **16:** 492–96.
10. Siesjö B. Lactic acidosis in the brain: Occurrence, triggering mechanisms, and pathophysiological importance. In: Porter R, Lawrenson G, eds. Metabolic acidosis (Ciba Foundation symposium no 87). London: Pitman Books, 1982: 77–100.
11. Myers RE. Lactic acid accumulation as a cause of brain oedema and cerebral necrosis resulting from oxygen deprivation. In: Korobkin R, Guilleminault G, eds. Advances in perinatal neurology. New York: Spectrum, 1979: 85–114.
12. Rehncrona S, Rosen I, Siesjö B. Excessive cellular acidosis: an important mechanism of neuronal damage in the brain. *Acta Physiol Scand* 1981; **110:** 435–47.
13. Edgren E, Terent A, Hedstrand U, Ronquist G. Cerebrospinal fluid markers in relation to outcome in patients with global cerebral ischaemia. *Crit Care Med* 1983; **11:** 4–6.
14. Jordan GW, Statland B, Halsted C. CSF Lactate in diseases of the CNS. *Arch Intern Med* 1983; **143:** 85–87.
15. Brook I, Bricknell KS, Overturf GD, Finegold SM. Measurement of lactic acid in cerebrospinal fluid of patients with infections of the central nervous system. *J Infect Dis* 1978; **137:** 384–90.
16. Menkes JH. Improving the long term outlook in bacterial meningitis. *Lancet* 1979; ii: 559–60.
17. Sherman IW. Biochemistry of *Plasmodium* (malaria parasites). *Microbiol Rev* 1979; **43:** 453–95.
18. Jensen MD, Conley M, Helstowski LD. Culture of *Plasmodium falciparum*: The role of pH, glucose, and lactate. *J Parasitol* 1983; **69:** 1060–67.
19. Pfaller MA, Krogstad DJ, Parquette AR, Nguyen-Dinh P. *Plasmodium falciparum*: Stage-specific lactate production in synchronized cultures. *Exp Parasitol* 1982; **54:** 391–96.

References continued at foot of next column

TRANSFER OF IgA DEFICIENCY TO A BONE-MARROW-GRAFTED PATIENT WITH APLASTIC ANAEMIA

LENNART HAMMARSTRÖM BERIT LÖNNQVIST
OLLE RINGDÉN C. I. EDVARD SMITH
THOMAS WIEBE

Departments of Clinical Immunology, Medicine, and Transplantation Surgery, Huddinge Hospital, Huddinge, Sweden; Department of Immunology, Wallenberglaboratory, Lilla Frescati, Stockholm; and Department of Paediatrics, Lunds University Hospital, Lund, Sweden

Summary IgA deficiency developed in a 2-year-old boy with aplastic anaemia who received a bone-marrow graft from his HLA-identical, 6-year-old, IgA-deficient sister. Southern blot analysis revealed the presence of α-genes in both children, thus suggesting a defect of lymphocyte stem-cell differentiation as a cause of IgA deficiency. Tissue typing showed homozygosity of HLA A1, B8, DR3, the haplotype associated with IgA deficiency in healthy people. Despite normal serum levels of IgG subclasses in both donor and recipient, both children showed a relative lack of specific IgG2 anticarbohydrate antibodies. This suggests that their IgA deficiency is part of a more fundamental aberration of immunoglobulin class and subclass distribution.

Introduction

SELECTIVE IgA deficiency (serum IgA <0·05 g/l) is the commonest immunodeficiency in man.[1] Genetic factors clearly influence the serum levels of IgA, and deficiency appears to be associated with genes within the HLA locus.[2,3] In a number of cases lack of IgA is associated with concomitant IgG2 deficiency,[4] a prognostic marker for permanent lack of IgA.[5]

IgA synthesis is highly dependent on thymus-derived cells,[6] but it is still unclear whether the impaired capacity seen for differentiation in IgA-deficient individuals is due to altered thymic/bursal microenvironment or reflects a genetically determined defect in the maturation of T or B cells and/or a structural defect of IgA heavy-chain constant genes.

Bone-marrow transplantation is a well-established method for treatment of patients with haematological malignancies, severe aplastic anaemia, and some inborn errors of metabolism. We describe a patient with aplastic anaemia who received a bone-marrow graft from his IgA-deficient sister.

N. J. WHITE AND OTHERS: REFERENCES—*continued*

20. White NJ, Warrell DA, Chanthavanich P et al. Severe hypoglycaemia and hyperinsulinaemia in falciparum malaria. *N Engl J Med* 1983; **309:** 61–66.
21. MacPherson G, Warrell MJ, White NJ, Looareesuwan S, Warrell DA. Human cerebral malaria: a quantitative ultrastructural analysis of parasitised erythrocyte sequestration. *Am J Pathol* (in press).
22. Warrell DA, Looareesuwan S, White NJ, et al. Permeability of the blood-CSF-barrier in human cerebral malaria. Abstract. *XI Int Cong Trop Med Malaria.* Calgary, Canada, Sept 16–22, 1984.
23. Weyne J, Leusen I. Lactate in CSF in relation to brain and blood. In: Cserr HF, ed. Fluid environment of the brain. New York: Academic Press, 1975: 255–76.
24. Plum F, Posner JB. Blood and cerebrospinal fluid during hyperventilation. *Am J Physiol* 1967; **212:** 864–70.
25. Chapman AG, Meldrum BS, Siesjö B. Cerebral metabolic changes during prolonged epileptic seizures in rats. *J Neurochem* 1977; **28:** 1025–35.
26. Krishna S, Shoubridge EA, White NJ, Weatherall DJ, Radda GK. *Plasmodium yoelii*: Blood oxygen and brain function in the infected mouse. *Exp Parasitol* 1983; **56:** 391–96.
27. Gardiner M, Smith ML, Kagstrom ML, Shohami E, Siesjö BK. Influence of the blood glucose concentration on brain lactate accumulation during severe hypoxia and subsequent recovery of brain energy metabolism. *J Cereb Blood Flow Metab* 1982; **2:** 429–38.

IgA-deficiency subsequently developed in the recipient, suggesting a stem-cell defect in IgA deficiency.

Methods

Case-history

This boy, born in August, 1980, after 40 weeks' gestation, had been well apart from recurrent otitis media and obstructive bronchitis during the second year of life. In September, 1982, he was admitted to hospital with a 3-week history of fever, pallor, and multiple haematomas. Anaemia (haemoglobin 6·3 g/dl), leucopenia ($0·44 \times 10^9$/l), and thrombocytopenia ($<10 \times 10^9$/l) suggested aplastic anaemia, which was confirmed by a bone-marrow sample showing a total lack of myelopoiesis and thrombopoiesis. No underlying cause of the pancytopenia was found. Treatment with steroids was immediately initiated and continued for 1 month. Since no therapeutic effects were achieved, he was transplanted in November, 1982, with bone marrow from his blood-group (O, rhesus positive) and HLA (A1, B8, DR3) identical, mixed-lymphocyte-culture-negative 6-year-old sister ($5·1 \times 10^8$ bone marrow cells/kg body-weight). He was given intravenous cyclophosphamide (50 mg/kg body weight) preoperatively for 4 days. Postoperatively he was treated for 1 year with cyclosporin to prevent rejection and graft-versus-host disease. 1 year after transplantation he was vaccinated with 'Pneumovax' (containing the polysaccharides against which antibody levels were subsequently tested). At present he is well without any medication and the bone marrow is totally repopulated.

Immunoglobulin Determinations

Serum immunoglobulin class and subclass levels were determined in immunodiffusion (IgM, IgG, IgA), enzyme-linked immunosorbent assay (ELISA) (IgD, IgA1, IgA2), or radio-immunoassay (IgE) with commercially available reagents. Serum levels of IgG subclasses were measured by immunodiffusion (kindly performed by Dr V. Oxelius, Lund, Sweden). The IgG subclass distribution of antigen-specific antibodies was analysed in ELISA as described previously.[7,8] Anti-IgA antibodies were measured in a haemagglutination assay using human red cells (O, rhesus negative) coated with purified serum IgA.[9] The presence of IgA heavy-chain constant genes was determined on Southern blots on Pst I digested leucocyte DNA using the α2XP8 probe (kindly supplied by Prof T. Rabbitts, Cambridge).[10]

Tissue Typing

The trypan-blue exclusion test was used for HLA A, B, C, and DR typing. Cell surface markers were identified and functional tests were carried out as described elsewhere.[11]

Results

Immunoglobulins (Table I)

Before transplantation the patient's immunoglobulin levels were essentially normal. The donor was deficient in IgA. Both parents had normal immunoglobulin levels. 16 months after transplantation, the now 8-year-old donor remains IgA deficient with a normal IgG subclass distribution. The recipient gradually lost serum IgA after transplantation and has remained IgA deficient (fig 1), whereas the IgG subclass distribution remains normal. Anti-IgA antibodies could not be detected in either the donor or the recipient after transplantation. Both IgA heavy-chain constant genes were

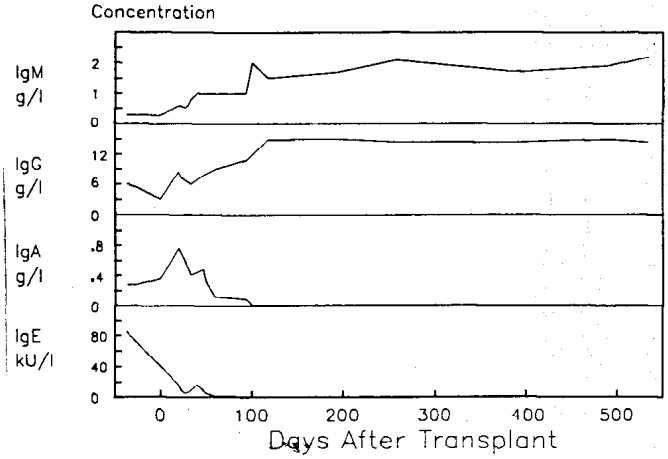

Fig 1—Immunoglobulin levels in a 2-year-old boy with aplastic anaemia after receiving bone-marrow graft from his 6-year-old IgA-deficient sister.

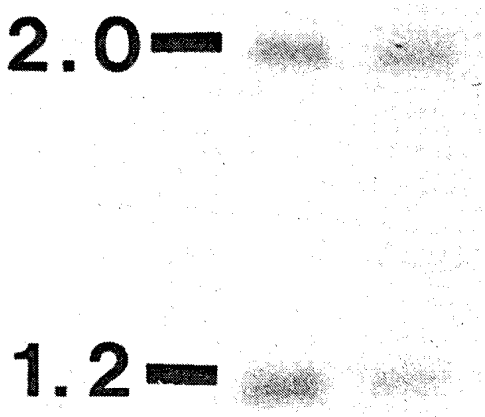

Fig 2— Southern blot (2XP8 probe on Pst 1 digests of leucocyte DNA) showing the two α-gene bands (2·0 kb and 1·2 kb).

Left lane, recipient 16 months after bone-marrow transplant; right lane, donor.

TABLE I—IMMUNOGLOBULIN LEVELS BEFORE AND AFTER TRANSPLANTATION

Subject	IgM	IgG (g/l)					IgA (g/l)			IgD (mg/l)	IgE (kU/l)
		Total	IgG1	IgG2	IgG3	IgG4	Total	IgA1	IgA2		
*Before transplantation:**											
Recipient	0·3	6·1	4·56	1·23	0·26	<0·01	0·3	0·27	0·08	0·03	82
Donor	1·3	11·9	..	..	..	..	<0·001	..	..	0·04	..
After transplantation:†											
Recipient	1·8	9·8	8·16	1·13	0·52	<0·01	<0·001	..	..	7·6	2·4
Donor	1·2	11·9	9·58	2·00	0·29	<0·01	<0·001	..	..	4·8	5·5
Mother	1·0	10·7	..	..	..	..	1·8	..	..	10·0	3·3
Father	1·1	9·6	..	..	..	..	2·8	..	..	10·5	46

*Samples obtained 1 month before transplantation. †Samples obtained 16 months after transplantation.

TABLE II—IgG SUBCLASS DISTRIBUTION OF ANTICARBOHYDRATE ANTIBODIES* AFTER TRANSPLANTATION

Antigen	Transplant subjects: Subject	Age (yr)	IgG1	IgG2	IgG3	IgG4	Controls (mean values)‡: No of subjects	Age (yr)	IgG1	IgG2	IgG3	IgG4
Teichoic acid†	Recipient	4	0·48	0·00	0·00	0·11	12	3–5	0·78	0·50	0·05	0·16
Teichoic acid	Donor	8	0·72	0·38	0·00	0·00	9	7–9	0·78	0·93	0·06	0·45
PPS 6A	Recipient	4	1·88	0·13	0·45	0·04	5	3–5	0·52	0·89	0·36	0·20
PPS 6A	Donor	8	0·35	0·21	0·08	0·02	5	7–9	0·29	1·36	0·11	0·20
PPS 19F	Recipient	4	1·73	0·08	0·23	0·05	5	3–5	0·76	0·63	0·59	0·37
PPS 19F	Donor	8	0·33	0·04	0·02	0·02	5	7–9	0·69	0·55	0·50	0·37
PPS 23	Recipient	4	1·82	0·10	0·43	0·04	5	3–5	0·50	0·63	0·22	0·12
PPS 23	Donor	8	0·38	0·06	0·02	0·01	5	7–9	0·48	1·28	0·12	0·17

PPS = pneumococcal capsular polysaccharide.
*Absorbance after 20 min (teichoic acid) or 30 min (PPS) incubation. †Polyribitol phosphate teichoic acid (type A β) from *Staph aureus*. ‡Data from Hammarström et al.[12]

present in the donor and in the recipient after transplantation, as shown on Southern blotting using an α-gene cDNA probe on Pst I digests of leucocyte DNA (fig 2).

Tissue Typing

The donor and recipient were both HLA (A1, B8, DR3) homozygous, a haplotype associated with IgA deficiency.[2,3] A remarkable degree of HLA sharing was observed in the parents, the father being HLA A1,2, B8,40, Cw3, DR3 and the mother HLA A1,2, B8,40, Cw3, DR3,8.

IgG Subclass Distribution of Specific Antibodies

A relative lack of IgG2 antibodies against a number of different polysaccharide antigens, including teichoic acid from *Staphylococcus aureus*, was found in both donor and recipient after transplantation (table II), compared with age-matched controls. The cells producing antibodies against teichoic acid, which are normally of the IgG2 subclass, are usually transferred during bone-marrow transplantation, and the recipients acquire the subclass distribution pattern of the donor.[12] A low level of anti-dextran B512 antibodies of the IgG2 subclass was found in the recipient before transplantation but none were found 16 months after transplantation or in the donor. Levels of specific IgG2 antibodies against a variety of pneumococcal capsular polysaccharides were also low when compared to age-matched controls[12] despite recent pneumococcal vaccination (table II).

TABLE III—LACK OF INDUCTION OF IGA SECRETION IN VITRO IN CELLS FROM AN IgA-DEFICIENT PATIENT*

Cell source†: T‡	B‡	Untreated: IgM	IgG	IgA	Pokeweed mitogen: IgM	IgG	IgA
Patient	Patient	0	0	0	230	140	0
Blood donor	Blood donor	0	0	0	800	220	140
Blood donor	..	0	0	0	0	0	0
..	Blood donor	0	0	0	0	0	0
Patient	Blood donor	NT	NT	NT	1000	760	80
Blood donor	Patient	NT	NT	NT	4500	1100	0

NT = not tested.
*Results are expressed as plaque-forming cells/culture (no of cells secreting a given Ig class).
†Cells were co-cultivated ($0·75 \times 10^6$ T-cell-enriched lymphocytes and/or $0·25 \times 10^6$ B-cell-enriched lymphocytes for 6 days in 1 ml RPMI 1640 medium supplemented with 10% heat-inactivated normal AB serum in round-bottom 5 ml tubes and either left untreated or stimulated with 10 μg/ml pokeweed mitogen.
‡T-cell-enriched suspensions were obtained by nylon-wool column purification, B-cell-enriched suspensions by depleting T cells by AET-SRBC rosetting.[11]

Surface Markers and Functional Studies

Before transplantation an almost total lack of T cells in blood and bone marrow was observed (<5% of total lymphocytes as measured by monoclonal antibodies Leu 2a and Leu 3a). The proportion of surface-immunoglobulin-positive cells remained normal or even increased (16–36%). The number of B cells was normal or low. A greatly increased proportion of blood lymphocytes were stained with the OKM1 monoclonal antibody (thought mainly to identify monocytes). 2 months after transplantation the proportion of T cells (measured as sheep-red-cell-rosetting cells) was normal (50%), with normal DNA-synthetic responses to various T-cell mitogens (phytohaemagglutinin, concanavalin A, pokeweed mitogen). The number of cells secreting immunoglobulin after pokeweed mitogen stimulation in vitro still remains slightly below normal 18 months after transplantation, with a lack of IgA-secreting cells (table III). Although T cells from the patient were capable of supporting differentiation of IgA-secreting cells from allogeneic blood donors, no IgA synthesis could be induced in the patient's B cells by stimulation with allogeneic normal T cells in co-cultivation experiments (table III) or Epstein-Barr virus, a direct B-cell activator.

Discussion

IgA deficiency is the most common immunodeficiency in man, with an estimated frequency of 1 in 500–1000.[1] It is still unclear whether the inability to secrete IgA is due to a stem-cell defect (with or without a concomitant deletion of IgA heavy-chain constant genes) or reflects a lack of differentiation due to an altered thymic/bursal microenvironment. The development of IgA deficiency in a previously normal recipient after bone-marrow transplantation with cells from an IgA-deficient donor suggests that the former is more likely. Gross deletions of genetic material coding for the IgA heavy-chain constant regions as a cause of IgA deficiency seem unlikely, since both genes were retained in leucocyte DNA, as shown by genomic blotting.

The capacity to produce IgA, as reflected by serum IgA levels, was retained up to 3 months after transplantation. Taking the estimated half-life of serum IgA (5 days) into account, these data suggest the persistence of recipient-derived cells during this period. The longevity of recipient cells after conditioning for bone-marrow transplantation has been suggested on the basis of persistence of isohaemagglutinins (mainly IgM)[13] and viral antibodies (mainly IgG)[14] 3–12 months after transplantation. When tested in vitro after transplantation, no IgA-secreting cells could be detected after stimulation of the patient's lymphocytes with a variety of

T-cell dependent or T-cell independent polyclonal B-cell activators, with or without the addition of purified T cells from normal allogeneic donors. T cells from the patient, however, were able to support differentiation of IgA-secreting cells when co-cultivated with allogeneic B cells in a pokeweed-mitogen driven system. Thus, a B stem-cell maturation defect appears to be the underlying defect in the patient.

Selective IgA deficiency is often associated with a relative or even total lack of IgG2.[4] In the donor-recipient pair described here, however, normal levels of IgG subclasses were found. Specific antibodies against carbohydrate antigens are normally mainly of the IgG2 subclass,[7] although in children substantial amounts of specific IgG1 antibodies are also formed.[8] Despite having normal IgG2 serum levels, however, both children showed a relative lack of specific IgG2 anti-carbohydrate antibodies. We have observed the same type of aberrant subclass distinction in occasional IgA-deficient adults (unpublished), which may suggest a defect more fundamental than a mere lack of IgA.

We thank Ms Lotta Tauson and Ms Charlotte Åman for skilful technical assistance. This work was supported by the Swedish Medical Research Council, the Petrus and Augusta Hedlund Foundation, and the Walter, Ellen and Lennart Hesselman Foundation.

Correspondence should be addressed to L. H., Department of Clinical Immunology (F79), Huddinge Hospital, S-141 86 Huddinge, Sweden.

REFERENCES

1. Hanson L-A, Björkander J, Oxelius V. Selective IgA deficiency. In: Chandra RK, ed. Immunodeficiency diseases. Edinburgh: Churchill Livingstone, 1984: 62–84.
2. Hammarström L, Smith CIE. HLA A, B, C and DR antigens in immunoglobulin A deficiency. *Tissue Antigens* 1983; **21:** 75–79.
3. Hammarström L, Axelsson U, Björkander J, Hanson L-A, Möller E, Smith CIE. HLA antigens in selective IgA deficiency. *Tissue Antigens* 1984; **24:** 35–39.
4. Oxelius V, Laurell A-B, Lindquist B, Golebiowska H, Axelsson U, Björkander J, Hanson L-A. IgG subclasses in selective IgA deficiency: Importance of IgG2-IgA deficiency. *N Engl J Med* 1981; **304:** 1476–78.
5. Chandra RK, Sahni S, Dearloue J, et al. Clinical and immunological studies of children with selective IgA deficiency. *Afr J Clin Exp Immunol* 1982; **3:** 281–92.
6. Elson CO. T cells specific for IgA switching and for IgA B-cell differentiation. *Immunol Today* 1983; **4:** 189–90.
7. Hammarström L, Granström M, Oxelius V, Persson MAA, Smith CIE. IgG subclass distribution of antibodies against *S aureus* teichoic acid and α-toxin in normal and immunodeficient donors. *Clin Exp Immunol* 1984; **55:** 593–601.
8. Freijd A, Hammarström L, Persson MAA, Smith CIE. Specific antibody levels in otitis-prone children. I. An analysis of plasma anti-pneumococcal antibody activity of the IgG class and subclasses. *Clin Exp Immunol* 1984; **56:** 233–38.
9. Hammarström L, Persson MAA, Smith CIE. Anti-IgA in selective IgA deficiency: In vitro effects and IgG subclass pattern of human anti-IgA. *Scand J Immunol* 1983; **18:** 509–13.
10. Flanagan JG, Rabbitts TH. Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε and α genes. *Nature* 1982; **300:** 709–13.
11. Hammarström L, Holm G, Palmblad J, Persson MAA, Smith CIE. Lack of IgG in a healthy adult: A rare case of dysgammaglobulinemia with undetectable serum IgG, IgA2 and IgE. *Clin Immunol Immunopathol* 1984; **30:** 1–10.
12. Hammarström L, Granström M, Möllby R, Oxelius V, Persson MAA, Smith CIE. Ontogeny of IgG2 antibodies against S aureus teichoic acid in normal and immunodeficient children. *Acta Paediatr Scand* (in press).
13. Bensinger W, Buchner CD, Thomas ED, Cliff RA. ABO-incompatible marrow transplants. *Transplantation* 1982; **33:** 427–29.
14. Wahren B, Gahrton G, Linde A, Ljungman P, Lönqvist B, Ringdén O, Sundqvist V-A. Transfer and persistence of viral antibody producing cells in bone marrow transplantation. *J infect Dis* (in press).