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Association Between HLA-A1, B8 in Children with Extrinsic Asthma and IgA Deficiency

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Abstract. The tissue types, immunoglobulin levels, and the presence of circulating autoantibodies were investigated in 57 children. Fifteen of these children suffered from bronchial asthma and, in addition, had no or very little IgA in their serum and saliva (Group 1 patients). Another fifteen children with asthma but normal immunoglobulin levels in serum and saliva (Group 2 patients), seven patients with selective IgA deficiency but without allergic diseases (Group 3 patients), and twenty healthy children served as controls. Sixty per cent of the Group 1 patients had the phenotype HLA-A1, B8, whereas this tissue type was found only in 27, 14 and 15 per cent, respectively, of the Group 2 and Group 3 patients and the healthy children. Furthermore, high IgM- and IgE levels were observed in most Group 1 patients, and in five of these patients (33 per cent) autoantibodies were present in the serum. In addition, eczema and glomerulonephritis occurred rather frequently in this group of patients. Conversely, normal immunoglobulin levels and absence of circulating autoantibodies were found in the remaining three groups of children. The results emphasize the heterogeneity of the IgA deficiency syndrome, and the question is raised as to whether the tissue type HLA-A1, B8 observed in most Group 1 patients reflects the abnormal immune reactivity of these patients.

Key words: HLA-A1, B8 tissue type – Circulating autoantibodies – IgA deficiency – Asthmatic children.

Introduction

Selective IgA deficiency is not a homogeneous syndrome. Indeed, many aetiological factors may by responsible and clinical symptoms may be absent or very diverse. In some cases the defect is inherited as an autosomal recessive characteristic and may be associated with childhood infections (Ammann and Hong, 1970; Buckley, 1975; Stocker et al., 1968). However, no causative factors can usually be

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detected, and the defect may even be transitory (Lawton et al., 1973; Østergaard, 1977b).

IgA deficiency, absolute or relative, has often been reported in children and adults with asthma and atopic dermatitis (Kaufman and Hobbs, 1970; Buckley, 1975; Østergaard, 1977a). However, most patients with allergic diseases have normal immunoglobulin levels in their serum and secretions. The aim of the present study was to investigate whether children with asthma and selective IgA deficiency might have certain common tissue types, unlike children with asthma but normal immunoglobulin levels, non-allergic children with selective IgA deficiency, and normal children.

Materials and Methods

Patients. The series was comprised of 37 patients, four to fourteen years of age, who were divided into 3 groups: (1) 15 patients with a median age of $8^{1}/_{12}$ years, who suffered from bronchial asthma and, in addition, had no or very little IgA in their serum and saliva, (2) 15 consecutive patients with bronchial asthma but normal immunoglobulin levels (median age $7^{8}/_{12}$ years) and (3) seven non-allergic children with selective IgA deficiency (median age $9^{9}/_{12}$ years). These patients are henceforth referred to as Group 1, Group 2 and Group 3 patients.

In all the patients with asthma, the diagnosis of allergy was based on a positive case history, positive prick tests and the demonstration of allergen specific IgE antibodies in serum by the RAST technique (Phadebas kit, Pharmacia, Copenhagen). All the seven non-allergic children with selective IgA deficiency had negative prick tests to an extensive test panel of allergens as well as negative RAST tests. Furthermore, none of these patients had eosinophilia in the peripheral blood. The main problems of the seven Group 3 patients were recurrent and chronic respiratory infections, but in one of these patients acute hepatitis developed, probably due to halothane anaesthesia. This patient had also been treated for three years with phenytoin because of repeated seizures following a skull fracture.

Controls. Twenty healthy children from the same geographical area as the patients were selected with regard to age to match the patients. None of the controls had experienced recurrent infections or atopy, and there was no evidence of allergic diseases among their first degree relatives. The median age of the controls was $8^{4}/_{12}$ years. None had eosinophilia in the peripheral blood, and all had negative RAST tests to pollen, animal danders, moulds, food stuffs and house dust mite.

Serum and Saliva Immunoglobulin Assays. Serum and saliva IgG, IgA and IgM levels in the patients and controls were determined by a modified electroimmuno technique using carbamylation of the samples prior to electrophoresis (Weeke, 1968). The method, which has been described in detail elsewhere (Østergaard, 1977a), allowed the detection of IgA in serum at a concentration of 2 mg per 100 ml and in saliva at a concentration of 0.77 mg per 100 ml. Serum IgE was measured with a radioimmunosorbent test (PRIST, Pharmacia, Copenhagen). The sensitivity for detection of IgE in serum by this method was 5 IU/ml.

Assay for T-cell Determination. The method used will be summarized since it has already been published elsewhere (Østergaard and Eriksen, 1977). Lymphocytes were harvested from peripheral blood on a Ficoll-Isopaque gradient and re-suspended in RPMI medium (Biocult, Glasgow) supplemented with penicillin, streptomycin and glutamine. This mixture will henceforth be referred to as medium.

The determination of T-cells was performed according to Jondal et al. (1972). Equal volumes of lymphocytes (3×10^6/ml) pretreated with papain (Merck), and sheep red blood cells (SRBC) (4% in Hank's balanced salt solution) were incubated at 37° C for 30 min. The mixture

was centrifugated and kept overnight in an ice bath. The percentages of erythrocyte rosette forming cells (E-RFC) were evaluated under a light microscope, and the samples were evaluated in duplicate. Lymphocytes with three or more surface bound SRBC were counted as rosettes.

Phytohemagglutinin (PHA) Stimulation of Peripheral Lymphocytes. The cell pellet obtained from the interphase layer was resuspended in medium supplemented with 20% heat-inactivated human AB serum to a final concentration of 1.5×10^6 cells per ml. The cells were cultured in round-bottomed Linbro Is-Fb-96 microculture plates. Cultures were set up in triplicate with 3×10^5 cells per culture. PHA (Wellcome) 0.2, 1.0 and 5.0 microgram per culture was added to the cultures. Cultures from the patients without PHA and cultures from the controls in the presence or absence of PHA served as controls. The microplates were incubated at 37° C in humidified air with 5% CO_2 for 72 h. Eighteen hours before termination of the culture period, 0.12 MicroCi ^{14}C-thymidine was added to each well. The cultures were terminated by placing them in an ice bath. Finally, they were harvested on glassfibre filtres and counted in a liquid scintillation counter (Packard).

Examination of Serum Autoantibodies. This was performed by the Autoimmune Laboratory, Statens Seruminstitut, Copenhagen, using a hemagglutinin technique. Serum was investigated for the presence or absence of the following human antigens: (1) parietal cell antigen, (2) adrenal cortex antigen, (3) thyroglobulin antigen, (4) smooth muscle antigen, (5) striated muscle antigen, (6) mitochondria antigen and (7) antinuclear factor (ANF). The results are expressed as 0 to ++++.

Assay for HLA-antigens was carried out by the Tissue Typing Laboratory, Aalborg Hospital North. The tissue typing was done by the microcytotoxicity test (Kissmeyer-Nielsen, 1977) on lymphocytes separated on the Ficoll-Isopaque gradient. The lymphocytes were tested against 113 antisera.

Statistical evaluation of the results was, in case of unpaired observations, performed by the Mann-Whitney test. In a few instances a chi-square test was done. The confidence limits of the observations were chosen at the 5 per cent level of significance.

Results

Serum and saliva immunoglobulin levels in the patients and controls are listed in Table 1. In eight Group 1 patients, IgA was not present either in serum or in saliva. In the remaining seven Group 1 patients, IgA was detectable in serum at concentrations from 6 to 24 mg per 100 ml (median 11 mg per 100 ml) and in saliva at concentrations from 0.82 to 1.11 (median 0.92 mg per 100 ml). Compared with a median of serum IgA of 78 and 88 mg per 100 ml in Group 2 patients and the healthy controls, respectively, the differences were highly significant ($P < 0.01$). With regard to saliva IgA, similar results were obtained ($P < 0.01$). The level of serum IgG in Group 1 patients was within the same range as that in the Group 2 and Group 3 patients and the healthy controls. Conversely, a significant increase in serum- and saliva IgM in Group 1 patients was observed, compared with the IgM levels in Group 2 and Group 3 patients and the healthy controls ($P < 0.01$; < 0.05; and < 0.01, respectively). Furthermore, a significant increase in serum IgE levels in Group 1 patients was found, compared with the serum IgE levels in the remaining three groups of children ($P < 0.05$; < 0.01; and < 0.05, respectively). No significant difference between the serum- or saliva IgA levels in Group 1 and Group 3 patients was observed.

The results of the T-cell assays appear in Table 2. In some Group 1 patients, a low number of E-RFC was observed. However, the difference from the Group 2

Table 1. Serum- and saliva Ig-levels in Group 1, Group 2 and Group 3 patients and their age-matched controls

Subjects	Serum Ig-levels			Saliva Ig-levels		
	IgG mg%	IgA mg%	IgM mg%	IgE IU/ml	IgA mg%	IgM mg%
Group 1						
Range	680—1250	0—24	88—490	140—4060	0—1.11	0—2.46
Median	1010	0	180	640	0	1.84
Group 2						
Range	720—1460	45—156	32—220	16—1014	1.9—7.2	0
Median	1140	78	88	230	5.3	0
Group 3						
Range	780—1520	0	36—192	11—240	0	0
Median	1030	0	101	72	0	0
Controls						
Range	830—1320	40—180	50—210	12—138	2.1—6.9	0
Median	980	88	91	55	4.9	0

Subjects	E-RFC (%)	PHA-stimulation (counts/min $\times 10^3$)
Group 1		
Range	44—78	0.43—59.6
Median	58.5	15.5
Group 2		
Range	64—81	17.0—49.6
Median	70.5	22.5
Group 3		
Range	58—85	14.5—40.6
Median	72.5	18.5
Controls		
Range	61—88	3.3—30.0
Median	71.5	17.5

Table 2. The number of E-RFC and the results of PHA stimulation of peripheral lymphocytes in Group 1, Group 2 and Group 3 patients and their age-matched controls

and Group 3 patients and the healthy controls was not significant. As regards the results of the PHA-stimulated cultures, subnormal counts were found in three Group 1 patients, but the median of counts/min in Group 1 patients was not significantly different from the medians observed in the other three groups of children.

The results of the *HLA-typing* and the investigation of *Autoantibodies* in Group 1 patients are given in Table 3. The phenotype HLA-A1, B8 was observed in nine of these patients (60 per cent). However, this phenotype was only found in

Table 3. The presence of allergic diseases and complicating disorders, the results of tissue typing, and the estimation of circulating autoantibodies in Group 1 patients

Subjects	Allergic diseases	Complicating disorders	HLA-types	Serum autoantibodies (0−++++)
1	Asthma eczema	None	A9,B5,B13,Cw3	Mitochondria (+)
2	Asthma	None	A1,B8	None
3	Asthma	None	A1,B8	None
4	Asthma eczema	Glomerulonephritis	A1,A25,B8,B18	Parietal (++)
5	Asthma	None	A2,B27,B40,Cw3	None
6	Asthma	None	A1,A2,B8,B12	Adrenal cortex (++)
7	Asthma eczema	None	A1,B8	Parietal (++)
8	Asthma rhinitis	None	A2,B40,B13,Cw3	None
9	Asthma eczema	Glomerulonephritis	A1,A2,B8,B12	Mitochondria (++)
10	Asthma eczema	None	A1,A25,B8,B18	None
11	Asthma	None	A2,B13	None
12	Asthma	None	A1,B8	None
13	Asthma rhinitis	None	A1,B8	None
14	Asthma	None	A9,B13	None
15	Asthma eczema	None	A9,A25,B12,Bw21	None

4 Group 2 patients (27 per cent), in 1 Group 3 patients (14 per cent) and in 3 of the healthy children (15 per cent). Furthermore, the occurrence of the phenotype HLA-A1,B8 among healthy, white, adult blood donors tested at our laboratory was 18.5 per cent, and the frequency of this phenotype in Group 1 patients was significantly different from the phenotypes observed in Group 2 and Group 3 patients and the healthy controls ($P < 0.01$; < 0.05; and < 0.01, respectively).

In five Group 1 children (33 per cent), circulating autoantibodies were present (Table 3). Conversely, autoantibodies were not found in either the Group 2 and Group 3 patients or in the healthy controls.Two of these five Group 1 patients had previously suffered from acute glomerulonephritis, and furthermore, in six Group 1 patients, asthma was complicated by eczema. The latter was the case in only one of the fifteen Group 2 patients.

Discussion

The polymorphic aspects of IgA deficiency suggest that multiple pathogenic mechanisms may be involved. Previous studies have reported normal numbers of circulating IgA B-lymphocytes in these patients. In addition, pokeweed mitogen often induced in vitro peripheral lymphocyte transformation to plasmocytes actively secreting IgA (Delespesse et al., 1976; Østergaard, 1977a).

HLA-A1,B8 is by far the most common haplotype found among caucasians, and this antigen combination has been found in healthy whites with a frequency

of 17 per cent (Thomson et al., 1976). However, in patients with asthma complicated by eczema, the tissue type antigen combinations A1 + B8 have been reported with a frequency of about 30 per cent (Turner et al., 1977).

In the present study, only Group 1 patients had elevated immunoglobulin levels, circulating autoantibodies and complicating diseases, presumably due to altered immune balance. Furthermore, these patients had a significantly increased incidence of the tissue type HLA-A1, B8. Increased levels of circulating autoantibodies have been reported in others patients with selective IgA deficiency (Ammann and Hong, 1970), and it has been suggested that the formation of autoantibodies in IgA deficient individuals may be related to increased absorption of macromolecules from the intestinal tract (Walker, 1976).

The tissue type HLA-B8 has often been found in diseases of autoimmune origin (e.g. thyroiditis, Addison's disease, myasthenia gravis and systemic lupus erythematosis) (Dausset et al., 1974), and several authors have claimed that B8 is a marker for high level antibody responses (Hors et al., 1974; Dausset and Hors, 1975). Furthermore, the number of B-cells generally reflects the state of immunoglobulin secretion, and a highly significant increase in circulating B-cells has been found among individuals with the tissue type HLA-A1 (Hors et al., 1974). Hence, the increased IgM and IgE levels and the presence of circulating autoantibodies may be the result of the phenotype HLA-A1, B8 found so frequently in Group 1 patients. This hypothesis may be supported by the observation of a lower incidence of the tissue type HLA-A1, B8 in the non-allergic patients with selective IgA deficiency on the one hand, and in the normoimmunoglobulinemic, allergic patients on the other, in whom neither circulating autoantibodies nor substantially elevated immunoglobulin levels were observed.

However, increased IgM levels are a common finding in individuals with selective IgA deficiency (Savilahti, 1973), and furthermore, it has been proposed that an increased production of autoantibodies may be due to a defective T-cell function (Moretta et al., 1976). In addition, there is now good evidence that a complex balance exists in T-cell helper and T-cell suppressor functions in the generation or "switch off", respectively, of IgE antibody responses (Ishizaka, 1976). These T-cell functions appear to be under the control of genes located within the major histocompatibility complex (Hamburger and Barazel, 1972; Levine et al., 1972).

In Group 1 patients, low and borderline numbers of T-cells, as well as low mitogenic responses after PHA stimulation of peripheral lymphocytes, were observed. However, as attempts to differentiate helper- and suppressor T-cells (Byrom et al., 1977) have not been performed, a possible increased T-cell helper activity or a decreased T-cell suppressor function influencing the IgE antibody formation in the Group 1 patients could not be established. However, a low number of circulating T-cells in children with asthma—often associated with eczema— has been reported earlier (Strannegård et al., 1976; Østergaard, 1977a).

Earlier investigations in patients with selective IgA deficiency have disclosed the heterogeneity of this syndrome (Buckley, 1975; Delespesse et al., 1976; Østergaard, 1977a). The results of the present study, as well as earlier studies in similar patients (Strannegård et al., 1976; Østergaard, 1977a), emphasize that a possible subgroup of children with extrinsic asthma and eczema associated with (1)

selective IgA deficiency (poor immune exclusion of environmental antigens), (2) low-normal T-cell function (insufficient suppression of IgE antibody responses) and (3) the phenotype HLA-A1, B8 (elevated and altered immune responses) may exist. So far, histocompatibility studies have been only minimally useful in this part of clinical medicine. However, they may provide important clues in clinical research into disease classification and mechanisms, but more extended studies in similar patients are needed before any firm conclusions can be drawn.

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