

## **Food antibodies, intestinal permeability and HLA status in IgA deficient blood donors identified by a new rapid screening test**

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### **Summary**

Circulating food antibodies, intestinal permeability and HLA status were studied in twelve blood donors previously identified as being selectively IgA deficient from screening 10 000 donations by means of a latex agglutination inhibition test, and confirmed by laser nephelometry. Assessment of intestinal function also included clinical history and standard laboratory tests. The donors proved to be healthy with no evidence of autoimmune disease or allergy. Nine donors (75%) had precipitins to food antigens. None had increased intestinal permeability as measured by the cellobiose/mannitol absorption test. HLA-A, B and DR antigen distribution was normal. IgG sub-class distribution was normal with the exception of an IgG2 level slightly below normal in one donor (0.9 g/l). An unexpected finding was a return of IgA levels to normal in four donors (33%) within 6 months.

### **Introduction**

Selective IgA deficiency is the commonest immunodeficiency syndrome, its prevalence varying in different populations from 1 in 328 [1] to 1 in 3040 [2], the average value being around 1 in 700. Many instances of familial IgA deficiency have been reported [3], and several studies have shown a significant association between IgA deficiency and HLA B8 [3–6], although no clear pattern of inheritance has emerged [3].

There is a well-established association between selective IgA deficiency and autoimmune diseases [7, 8], atopic allergy [9] and various gastrointestinal disorders such as coeliac disease and Crohn's disease [10]. It is not known whether these associations have a common genetic basis linked to the HLA B8 phenotype, or whether IgA deficiency itself may predispose to the development of these diseases by some as yet undiscovered mechanism. Several studies have indicated that IgA-deficient subjects more commonly have circulating antibodies to milk proteins than the normal

population [7, 11, 12] without attempting to relate these findings to intestinal structure or function, or to other associated diseases. Intestinal diseases such as Crohn's disease and coeliac disease are themselves associated with an increased frequency of serum food antibodies [13], and the interpretation of food antibodies in IgA deficient subjects is, therefore, dependent upon the demonstration of normal small bowel structure and function.

The studies reported here formed part of a project in which active systemic immunity to food antigens in healthy blood donors was examined. IgA-deficient donors were identified by means of a previously reported rapid screening test based on inhibition of latex agglutination [14], and confirmed by laser nephelometry. Food antibodies in these donors was studied in relation to HLA status and blood groups, and assessed clinically for evidence of small bowel disease, atopy and autoimmune diseases. In addition to standard clinical and laboratory methods, the cellobiose/mannitol sugar permeability test was used as a means of screening for small intestinal dysfunction.

## Subjects and methods

### *Subjects*

Seventeen IgA-deficient blood donors were identified by means of a previously described latex agglutination inhibition test for IgA, which permits rapid screening of large numbers of sera. Briefly, 5  $\mu$ l sheep anti-human IgA (from the Scottish Antibody Production Unit, Law Hospital, Carlisle) is added to 35  $\mu$ l of serum in a microtitre plate and incubated for 30 min at room temperature. Twenty  $\mu$ l are transferred to a well in a larger plate and 28  $\mu$ l of IgA-coated latex added, and this plate is agitated gently for 15 min. Sera with less than 0.5 g/l IgA result in latex agglutination by anti-IgA. The latex was coated with IgA obtained from myeloma serum, by incubation in a 0.1 M glycine-saline buffer, pH 8.2, at 60°C for 20 min. Anti-IgA activity is also detected by this test system.

10 045 serum samples from blood donors were screened and sera which showed agglutination in the test had the IgA content measured by laser nephelometry [15]. Eighteen samples, representing seventeen donors (one donor gave blood twice during the study period), had an IgA concentration of less than 0.5 g/l. These donors were contacted by letter and asked if they would agree to attend for interview, further blood tests, and the test of intestinal permeability described below. Twelve agreed to participate. These donors were interviewed, and the aims and methods of the study were explained in detail. All gave written consent to further studies.

Clinical assessment of the donors consisted of a questionnaire designed to elicit a history of infection, allergy, autoimmunity or gastrointestinal disease, and a family history was taken. Blood was obtained for repeat IgA estimation and IgG and IgM by laser nephelometry, IgE by radioimmunoassay, full blood count and blood film, folate B12, iron and IBC, proteins and electrolytes including calcium, and for the estimation of food antibodies and HLA status. IgG sub-classes were measured by radial immunodiffusion using the method described by French & Harrison [16].

### *Food antibodies*

Serum samples were tested by a microprecipitation method for antibodies to milk, fish, wheat flour, egg yolk, chicken, pork and foetal calf serum [13]. In a previous study, this

method identified food antibodies in 45% of patients with eczema and food allergy, 15% of patients with eczema alone, and 15% of patients with asthma and rhinitis; and in an unpublished study of normal blood donors we found food antibodies (usually to milk protein) in 4%.

#### *Cellobiose/mannitol sugar permeability test*

This test of intestinal permeability and its relationship to jejunal histology has been described in detail elsewhere [18]. After an overnight fast the subject voids his or her bladder and ingests a 150 ml solution containing 2 g mannitol, 5 g cellobiose, 20 g lactose and 20 g sucrose in water (osmolality approximately 1500 mosm). All urine passed within 5 hr of the sugar drink is collected, the volume measured and an aliquot stored at  $-20^{\circ}\text{C}$ . Mannitol in urine was measured by the method of Corcoran and Page [19], cellobiose by a method developed in this laboratory [18], and the results expressed as a ratio of percentage recovery of cellobiose to percentage recovery of mannitol.

#### *HLA typing*

HLA typing for HLA A, B, C and Dr antigens was performed using a standard microcytotoxicity test [20].

### Results

Four of the twelve donors who were initially found to be IgA deficient in the donor screening study had IgA levels within the normal range when tested 6 months later as part of the present study. There were also considerable variations in IgA levels in those remaining below the lower limit of normal for this laboratory (Table 1).

There were eight male (age range 21–41 years) and four female donors (age range 28–65 years). All of the donors were well at the time of the study. One donor gave a history of significant recurrent infection (sinusitis and otitis media), and two had mild allergies in the past. None had confirmed autoimmune disease and none had significant

**Table 1.** Food antibodies, blood groups and HLA status in IgA deficient blood donors

| Donor | IgA* (g/l) |       | Precipitins† | Blood group (ABO/Rh) | HLA-A  | HLA-B              | HLA-CW   | HLA-DR |
|-------|------------|-------|--------------|----------------------|--------|--------------------|----------|--------|
|       | 1          | 2     |              |                      |        |                    |          |        |
| 1     | 0.46       | 0.13  | M            | B neg                | 24, 29 | 14, W62, BW6       | X        | 3, 4   |
| 2     | 0.48       | 4.24  | M            | O pos                | 2, 3   | W44, W62, BW4, BW6 | X        | —      |
| 3     | 0.23       | <0.12 | M            | O neg                | 1, 30  | 13, W57, BW4       | 6        | 7      |
| 4     | 0.30       | 1.38  | O            | O pos                | 1, 3   | 7, 8, BW6          | 7        | 1, 3   |
| 5     | 0.46       | 1.95  | O            | O pos                | 29, 32 | W60, W44, BW4, BW6 | 3        | 7      |
| 6     | 0.38       | 2.01  | M, P, C, F   | A pos                | 11, 32 | 27, 35, BW4, BW6   | CW4, CW5 | 7      |
| 7     | 0.36       | <0.12 | O            | O pos                | 1, 2   | 7, W60, BW6        | X        | —      |
| 8     | 0.17       | <0.12 | M, F, CS     | A pos                | 2, 11  | 7, 8, BW6          | 7        | 4      |
| 9     | 0.12       | <0.12 | M, Y         | A pos                | 2, 28  | 7, 44, BW4, BW6    | 5, 7     | 1, 7   |
| 10    | 0.17       | <0.12 | M            | A pos                | 3, W32 | 8, BW6             | 7        | 3      |
| 11    | 0.49       | <0.12 | M            | A pos                | 1, W32 | 8, W49, BW4, BW6   | 7        | 1, 2   |
| 12    | 0.17       | <0.12 | M            | O pos                | 3, W32 | 35, W44, BW4, BW6  | 4, 5     | 1, 4   |

\* IgA measured by laser nephelometry; 6-month interval between 1 and 2.

† Serum precipitins to milk (M), fish (F), wheat flour (W), egg yolk (Y), chicken (C), pork (P) and foetal calf serum (CS).

gastrointestinal symptoms. Five subjects gave a family history of allergy, and two had a family history of rheumatoid arthritis. IgE levels were normal in all subjects (normal range 7.5–200 IU/ml). IgG sub-classes were normal, with the exception of one donor who had an IgG2 level of 0.94 g/l (normal range 1.0–7.25 g/l).

Two subjects showed biochemical evidence of iron deficiency (plasma iron 6 and 7  $\mu\text{mol/l}$ , respectively, TIBC 68 and 83  $\mu\text{mol/l}$ ), and one of these had microcytosis. Haemoglobin levels were invariably normal, even in the iron deficient donors, and no abnormalities were found in the other haematological and biochemical parameters tested.

Nine of the twelve subjects (75%) had precipitins to food antigens. All nine had antibodies to cows' milk protein, two had antibodies to fish and one each to chicken, pork, egg yolk and foetal calf serum. There was no correlation between precipitin positivity and IgA level. Intestinal permeability was normal in all subjects. The distribution of HLA-A, B and DR antigens was not significantly different from a control population evaluated previously [21].

## Discussion

The group of IgA-deficient subjects reported in this study were identified by screening 10 000 blood donors. In using blood donors for this type of study it was important to acknowledge the ethical constraints when screening normal volunteers, especially when the findings are not necessarily of benefit to the individual. Taken into consideration was the possible benefit to the service in having identified IgA-deficient donors available for the provision of blood and blood products to recipients with anti-IgA antibodies, and arrangements were made to have any donors suspected of having coeliac disease or other diseases assessed by an independent physician after first consulting that individual's own doctor. Most previous studies of food antibodies in IgA-deficient subjects have used patients, rather than individuals drawn from the 'normal' population.

In the event clinical and laboratory assessment of this group of IgA-deficient blood donors showed that they were in good health, with only one individual of the twelve reporting significant recurrent infection. No unusual findings emerged with respect to family history, ABO blood group or HLA status. Thus, it was not possible to confirm the reported association of IgA-deficiency with HLA B8 [4–6].

Antibodies to common foodstuffs were found in 75% of the subjects. This was a higher percentage than in most other reports [7, 11, 12], but in these previous studies only milk antibodies have been sought. In view of the known association between IgA-deficiency and coeliac disease, it is of interest that no antibodies to wheat flour were detected, though the significance of anti-gliadin antibodies in the pathogenesis of coeliac disease is in any case far from clear.

This increased incidence of food antibodies in IgA-deficient subjects could not be attributed to an abnormality of intestinal permeability as measured by the cellobiose-mannitol absorption test. This test has provided evidence of abnormal permeability not only in conditions associated with structural damage to the intestinal mucosa, such as coeliac disease and Crohn's disease, but also in patients, notably those with atopic eczema, shown to have normal jejunal mucosae [18]. However, it was not possible to rule out the possibility that IgA-deficient subjects may absorb abnormally large quantities of specific proteins that would normally be blocked by secretory IgA.

Nevertheless, the absence of a generalized defect in intestinal permeability suggested the possibility that the high levels of food antibodies found in association with IgA deficiency may be the result of a defect in immunoregulation.

The fluctuation in IgA levels in these subjects within a 6-month period was an unexpected finding, with four of the original twelve showing normal values on repeat testing. Only one longitudinal study of IgA levels has been reported [22], and the authors found a degree of fluctuation in IgA level which was greater than the variability inherent in the methods used for testing. The findings in this study confirm this, and indicate that caution is required in labelling individuals as IgA-deficient.

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