

Lack of Association between IgA Deficiency and Respiratory Atopy in Young Male Adults

A. FRANCO¹, R. PARRELLA¹, F. MURRU², P.R.J. AMES³, F. MARTUCCI¹,
G. ROTIROTTI⁴, P.V. MANFRONI², D. CIOFFI², C. TOMMASINO⁵ and V. ESPOSITO¹

¹Department of Infectious Diseases, Hospital "Dei Colli", Naples, Italy;

²Italian Air Force Health Service, Italy;

³Immunoclot Ltd, Leeds, U.K.;

⁴Department of Respiratory Allergy, Royal Brompton Hospital, London, U.K.;

⁵Department of Clinical Pathology, "S.Gennaro" Hospital, Naples, Italy

Abstract. *Background:* The issue of atopy and increased serum IgE in IgA deficiency is still a matter of debate. The aim of this study was to evaluate the prevalence of IgA deficiency and its relationship with respiratory atopy. *Materials and Methods:* A retrospective study on 4700 consecutive young males (age range 18-23), who underwent a health screen for admission to the Italian Airforce Academy between 1993 and 1995 was conducted. Serum IgA was measured by immunoturbidimetry and total and specific IgE by fluorescent enzyme immunoassay (Phadiatop FEIA, Pharmacia Cap System). Airway responsiveness was assessed by methacholine challenge. *Results:* IgA deficiency was detected in 0.34% (16/4700) subjects and atopy was detected in 8.6% (406/4700). The mean IgA was 243 mg/dl (95% CI 107, 442) in the 406 atopic subjects and 238 mg/dl (95% CI 100, 441) in 1544 controls. Only 6 (37.5%) of the IgA deficient subjects had subnormal IgE levels and 6 were positive in the fluorescent EIA. None of the IgA deficient patients presented with respiratory hyper-reactivity. *Conclusion:* Atopy is not more prevalent in young male adult IgA deficient subjects, who rather display a high frequency of recurrent sinusitis.

Selective IgA deficiency is the most common inherited immunoglobulin disorder characterized by serum IgA levels <5 mg/dl in the presence of normal or increased serum levels of IgG, IgM or IgE. Its prevalence ranges from 1:300 to 1:3000 according to the population under study (1). A figure of 0.35% was derived from apparently healthy young Italian male adults (2, 3) though it may be present in 2.66% of Caucasians. The

clinical significance of IgA deficiency is unclear (4) since it has been detected in healthy people as well as in patients with autoimmune (5) and atopic disorders (6, 7). The lack of IgA in serum is often associated with a reduced level of secretory IgA (8), but this does not seem to predispose to an increased frequency of infections unless a concomitant deficiency of IgG subclasses (particularly IgG2) is present. In this case infections may be more frequent and clinically more relevant (9, 10). Many studies have investigated the possible role of IgA deficiency and the development of allergic symptoms (11-13). To date the results are still very controversial. A possible role of IgA deficiency on the emergence of airway hyperresponsiveness (AHR) has also been suggested (14). The prevalence of IgA deficiency and its association with atopy and bronchial responsiveness was retrospectively evaluated in a large population of young male candidates to the Italian Air Force Academy between 1993 and 1995.

Patients and Methods

Patients. Consecutive young male adults (n=4700, age range 18-23 yrs) from different Italian regions underwent screening procedures for admission to the Italian Airforce Academy located in Naples (Italy) between 1993 and 1995.

The association between atopy and serum levels of IgA was evaluated by comparing two groups deriving from the original 4700 subjects. One group included the 406/4700 (8.6%) individuals found to have respiratory hypersensitivity according to serology, skin testing and AHR, the other included 1544/4700 (32.8%) individuals who proved negative to the same tests. The serum IgA levels were compared in the two groups. Controls were selected on the basis of a fluorescent enzyme immunoassay FEIA (<400 fluorescence units) and a negative history of allergic disorders.

Serum measurements. Serum IgA was measured by immunoturbidimetry (Roche Diagnostics, Basel, Switzerland) on a Cobas Mira S spectrophotometer (Roche Diagnostics). IgA deficiency was defined at a cut-off level below 5 mg/dl. Specific IgE were measured by a

Correspondence to: Dr. Vincenzo Esposito, MD, Ph.D., Department of Infectious Diseases, Hospital "Dei Colli", Via L. Bianchi, 80131 Naples, Italy. Tel/Fax: +39 0815908303, e-mail: esposvin@libero.it

Key Words: IgA deficiency, atopy, IgE, airway hyperresponsiveness.

fluorescent enzyme immunoassay (Phadiatop FEIA, Pharmacia Cap System – Pharmacia Diagnostics AB, Uppsala, Sweden) (15-17) that identifies reactivity towards the most common aero-allergens in Italy. To exclude patients with borderline results a positive FEIA was a value greater than 10000 FU.

In the IgA deficient patients (IgA <5 mg/dl) IgG and IgM levels were also measured by immunoturbidimetry (Roche Diagnostics, Basel, Switzerland) (reference values respectively 800-1700 mg/dl for IgG and 60-250 mg/dl for IgM). IgA deficiency was also confirmed by nephelometry (Behring Diagnostics, Deerfield, IL, USA). Antibodies against IgA were determined by agarose gel immunofixation (IFE, Paragon Beckman Inc., Brea, California, USA) employing serum from a patient with IgA myeloma.

To identify anti-IgA in patients with IgA deficiency, the IgA from the myeloma patient was run (100 volts, for 30 min, barbital buffer pH 8.6) in agarose gel and then immunofixed with control monoclonal anti-IgA and with serum from patients with IgA deficiency. Immunoprecipitating bands were identified after staining with Blue Paragon. B-lymphocytes expressing surface IgA were also quantified by Fluorescence Activated Cell Sorting (FACS) analysis (Epics Profile II, Coulter Inc. Hialeah, FL, USA) with monoclonal antibodies from Dako (Dako Denmark A/S, Glostrup, Denmark). Total and specific IgE were measured by enzyme-linked immunosorbent assay (Matrix Aeroplus, Abbott Laboratories, North Chicago, IL, USA), which allows identification of total and specific IgE against the most common aeroallergens (alternaria, penicillium, aspergillum, dog weed, betulla, olive, dog and cat dander, acarus domesticus, flour acarus, asenzio, lanciula, paritaria, ambrosia trifida and eliator and mouse tail).

In patients with a positive FEIA, respiratory atopy was confirmed by prick-tests. Anti-nuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA) and anti-endomysial antibodies were assayed by indirect immunofluorescence (GmbH Labordiagnostik, Leipzig, Germany). Complement fractions C3c, C4 and circulating immune complexes (CIC) were measured by nephelometry (Behring Diagnostics, Deerfield, IL, USA).

Pulmonary function. Forced expiratory volume in the 1st second (FEV₁) obtained with flow-volume equipment (Spirostar FG 90 – Werner Gut AG, Basel, Switzerland), was used as a measure of bronchial calibre; the best of 3 consecutive measurements was chosen.

Methacholine challenge. Methacholine was administered by a metered nebulizer dosimeter (MB3, Mefar, Brescia, Italy) connected to a mouthpiece and activated by patient inspiratory maneuver. Two solutions of lyophilic methacholine (0.2% and 1%) (Lofarma, Milan, Italy) were freshly prepared from powder preparations by dilution in distilled water. The dosimeter was activated for 0.6 sec to provide an output of 9.9 ± 0.25 µg/puff. After baseline measurements of FEV₁, phosphate buffer was inhaled (5 breaths), followed by cumulative doses of methacholine (from 20 to 2400 mg). FEV₁ was measured 1 min after phosphate buffer inhalation, and all subsequent changes in FEV₁ were compared with the postbuffer value. Inhalations were performed while the nose was clipped and consisted of a slow submaximal inspiratory maneuver starting at functional residual capacity. The test was discontinued if FEV₁ dropped >20% or if the highest dose of methacholine was reached. Dose-response curves were constructed by plotting the logarithmic value of the dose against the percent decrement in FEV₁. Provocative dose causing a 20% fall in FEV₁ (PD₂₀) was calculated by linear interpolation between the last 2 points.

Modifications of many other indices such as peak expiratory flow (PEF), maximal expiratory flow at 25% (MEF 25), 50% (MEF 50) and 75% (MEF 75) of the vital capacity were taken into account.

Statistical analysis. Fisher's exact test was employed to compare the prevalence of atopics (Phadiatop positives) in the normal population (4684 people) and in the 16 subjects with IgA deficiency. Reference values for serum IgA in the two populations selected (406 with respiratory hypersensitivity and 1544 not showing respiratory hypersensitivity derived from the total 4700 individuals) were calculated after the recommendations of the International Federation of Clinical Chemistry (18-20). The Refval program (dedicated software) built an empirical distribution curve (original data) whose fitness with a Gaussian distribution was determined by evaluation of skewness and kurtosis and by several statistical tests (Andersen-Darling, Kolmogorov-Smirnov, Kramer-von Mises). The program (21-23) automatically transforms data into exponential modular fashion if the distribution is significantly different from a Gaussian. This allows calculations of the reference interval in a parametric way. The mean serum IgA levels of the two groups were also compared by *t*-test for unpaired data.

Results

IgA deficiency occurred in 0.34% (16/4700) of the study population. Out of these subjects, 10/16 (62.5%) had a higher serum level of total IgG (associated with elevated CIC in two subjects), 2/16 (12.5%) had a low serum IgM (Table I) and 6/16 (37.5%) had IgE in the lower range of normal. The specific IgE measurement by enzyme-linked immunosorbent assay (Matrix Aeroplus, Abbott Laboratories) yielded 4 positive results whereas the more sensitive Phadiatop CAP System yielded 6 positive results (Table II). ANA, AMA, ASMA, anti-endomysial antibodies, anti-IgA antibodies, C3c, C4 and rheumatoid factor (RF) were negative or normal in all the IgA deficient subjects. By Fluorescence Activated Cell Sorting (FACS) analysis the total lymphocytes as well as B lymphocytes mounting surface IgA were within normal ranges. From the clinical standpoint, six IgA deficient subjects (37.5%) had recurrent sinusitis (all in non-tonsillectomised subjects). Patient No 4 had decreased hearing acuity at medium tones (3000 Hz). Amongst other problems, two patients had a thalassaemia trait and one a recurrent iron deficiency (Table III).

Phadiatop positivity was not different between the subjects with IgA deficiency as compared to the rest of the population ($p=0.5$) nor was the median serum IgA different between the atopic subjects and controls. In fact, the mean IgA was 243 mg/dl (95% CI 107, 442) in the 406 atopic subjects and 238 mg/dl (95% CI 100, 441) in the 1544 controls.

All the IgA deficient subjects were negative to methacholine challenge (PD₂₀ MCh > 3200 µg).

Discussion

This large survey confirmed a low prevalence of IgA deficiency (0.34%) in a male population homogeneous for age and representative of all the Italian regions (2). A higher, than normal,

Table I. Serum levels of IgA, IgG, IgM and circulating immune complexes (CIC) in 16 subjects with selective IgA deficiency (in bold subnormal values and in italics abnormal high values).

Cases	IgA mg/dl	IgG mg/dl	IgM mg/dl	CIC µcg/dl
1	4,0	1182	30	1,9
2	1,0	<i>3156</i>	106	<i>3,4</i>
3	1,0	<i>1935</i>	135	1,5
4	2,0	1556	179	1,7
5	3,0	<i>1907</i>	246	1,6
6	2,0	<i>2145</i>	157	1,6
7	3,0	1498	250	1,4
8	1,0	<i>2430</i>	160	<i>3,6</i>
9	1,0	<i>1950</i>	40	2,5
10	1,0	<i>2090</i>	110	1,9
11	4,0	980	210	1,8
12	2,0	1220	205	1,6
13	1,0	<i>2340</i>	117	1,6
14	3,0	<i>1960</i>	198	1,9
15	1,0	1640	241	1,5
16	1,0	<i>2190</i>	122	2,1

mean level of serum IgG in patients with IgA deficiency (2) was also confirmed, although it remains unclear whether this is a compensatory mechanism for IgA deficiency or whether a response to an increased tendency to infections (24-26).

None of the IgA deficient subjects had clinical or laboratory evidence of autoimmune disorders, nor were anti-IgA antibodies found in the IgA deficient subjects. However a 37.5 % prevalence of maxillary sinusitis occurred among the IgA deficient subjects confirming the essential role of secretory immunity in the control of upper airway infections (25). None of the IgA deficient subjects showed an increased level of IgE, rather 37.5% of them had low normal IgE levels, which could suggest some protection against recurrent respiratory tract infections according to some authors (26). Others have noted that serum IgA and IgE levels run parallel during lifetime (27).

Only six IgA deficient patients appeared positive with the Phadiatop screening test of which four were also positive in the Matrix system. Moreover, the percentage of positive Phadiatop tests among the normal (2024/4684, 43.2%) and IgA deficient subjects (6/16, 37.5%) were similar.

Phadiatop is a multi-FEIA for specific inhalant allergens that offers greater sensitivity (28-29) compared to the conventional Paper Radio Immuno Sorbent Test (PRIST) that measures only total IgE in detecting atopic subjects, and shows good correlation with Radio Allergo Sorbent Test (RAST) or skin tests (15-17, 30). Phadiatop is an important step forward in the field of mass screening programmes for inhalant allergies, hence it was chosen in this study for its fast turnaround time.

In this study the mean IgA levels were similar in both atopic and control subjects. Therefore this survey on a young male

Table II. Results of the screening test with the Phadiatop CAP system (FEIA) and specific IgE with Matrix AeroPlus Abbot.

Cases	Phadiatop	Matrix
1	523 (-)	Negative
2	15149 (+)	Negative
3	231 (-)	Negative
4	252 (-)	Negative
5	229 (-)	Negative
6	344 (-)	Negative
7	15234 (+)	Positive
8	12345 (+)	Positive
9	10670 (+)	Negative
10	234 (-)	Negative
11	267 (-)	Negative
12	376 (-)	Negative
13	11306 (+)	Positive
14	365 (-)	Negative
15	10320 (+)	Positive
16	213 (-)	Negative

Table III. Clinical characteristics of 16 subjects with IgA deficiency.

Cases	Tonsillectomized	Sinusitis	Other
1	No	Yes	Thalassaemia trait
2	No	Yes	No
3	No	Yes	Iron deficiency
4	No	No	Right hypoacusia
5	Yes	No	No
6	Yes	No	No
7	No	No	No
8	No	No	No
9	Yes	No	No
10	No	Yes	No
11	Yes	No	No
12	No	No	Thalassaemia trait
13	Yes	No	No
14	Yes	No	No
15	No	Yes	No
16	No	Yes	No

population did not support an association with respiratory atopy in IgA deficient subjects.

Finally, the data showed normal responsiveness to methacholine in subjects with IgA deficiency and concomitant atopy. Therefore no association was found between IgA deficiency, atopy and airway responsiveness. One limitation was certainly the low percentage of subjects with these two features. However, some authors have shown that IgA deficiency is not by itself a risk factor for the development of bronchial hyperreactivity. Moreover, similar tests have shown that airway hyperresponsiveness among children with IgA deficiency correlated with dust mite allergy, but not with general atopy (14).

In conclusion, an association between IgA deficiency, atopy and airway responsiveness is not demonstrated in young adult males. Further studies will be required on a larger population to acquire more information about this hypothesized association.

References

- Cunningham-Rundles C: Physiology of IgA and IgA deficiency. *J Clin Immunol* 21(5): 303-309, 2001.
- D'Amelio R, Fattorossi A, Berti R, Paganelli R and Castagliuolo PP: Serum IgG, IgA, IgM, IgE, salivary IgA levels and lung function in a healthy male population from the Italian Air Force: a preliminary study. *Ann Allergy* 53(5): 432-435, 1984.
- Weber-Mzell D, Kotanko P, Hauer AC, Goriup U, Haas J, Lanner N, Erwa W, Ahmaida IA, Haitchi Petnehazy S, Stenzel M, Lanzer G and Deutsch J: Gender, age and seasonal effects on IgA deficiency: a study of 7293 Caucasians. *Eur J Clin Invest* 34(3): 224-228, 2004.
- Mestecky J and Hammarstrom L: IgA-Associated Diseases. Mucosal Immune Defense Immunoglobulin A. Springer eBook Collection pp. 321-344, 2007.
- Aghamohammadi A, Cheraghi T, Gharagoziou M, Movahedi M, Rezaei N, Yeganeh M, Parvaneh N, Abolhassani H, Pourpak Z and Moin M: IgA deficiency: correlation between clinical and immunological phenotypes. *J Clin Immunol* 29(1): 130-136, 2009.
- Buckley RH: Clinical and immunologic features of selective IgA deficiency: Birth Defects Orig Artic 11(1): 134-142, 1975.
- Plebani A, Monafò V, Ugazio AG and Burgio GR: Clinical heterogeneity and reversibility of selective IgA deficiency in 80 children. *Lancet* 1(8485): 829-831, 1986.
- Hanson LA and Brandtzaeg P: Mucosal defence. In Immunologic Disorders in Infants and Children. 2nd Edition. RT Stihem, V Fulginiti (eds.). Philadelphia, Saunders, pp. 137-164, 1980.
- Oxelius VA, Laurell A, Lindquist B, Golebiowska H, Axelsson U, Björkander J and Hanson LA: IgG subclasses in selective IgA deficiency. Importance of IgG2-IgA deficiency. *N Engl J Med* 304(24): 1476-1477, 1981.
- Morell A, Muelheim E, Schaad U, Skvaril F and Rossi E: Susceptibility to infections in children with selective IgA and IgA-IgG subclass deficiency. *Eur J Paediatr* Aug 145(3): 199-203, 1986.
- Dellepiane RM, Pavesi P, Patria MF, Laicini E, Di Landro G and Pietrogrande MC: Atopy in preschool Italian children with recurrent respiratory infections. *Pediatr Med Chir* 31(4): 161-164, 2009.
- Bottcher MF, Haggstrom P, Björkstén B and Jenmalm MC: Total and allergen-specific immunoglobulin A levels in saliva in relation to the development of allergy in infants up to 2 years of age. *Clin Exp Allergy* 32(9): 1293-1298, 2002.
- Michel FB, Guendon R and Guerrero AJ: Serum IgE in patients suffering from IgA deficiency with or without atopy. *Nouv Presse Med* 5(29): 1811-1814, 1976.
- Papadopoulou A, Mermiri D, Taousani S, Triga M, Nicolaidou P and Priftis KN: Bronchial hyperresponsiveness in selective IgA deficiency. *Pediatr Allergy Immunol* 16(6): 495-500, 2005.
- Matricardi PM, Fattorossi A, Nisini R, Le Moli S, Castagliuolo PP and D'Amelio R: A new test for specific IgE to inhalant allergens (Phadiatop) in the screening of immediate respiratory hypersensitivity states. *Ann Allergy* 63(6 Pt 1): 532-535, 1989.
- Matricardi PM, Nisini R, Pizzolo JG and D'Amelio R: The use of Phadiatop in mass-screening programmes of inhalant allergies: advantages and limitations. *Clin Exp Allergy* 20(2): 151-155, 1990.
- Banker DD, Daftary VG, Daftary GV, Bhandari NM and Dayanand SR: Allergy diagnosis by pharmacocap system. *Indian J Med Sci* 53(9): 387-389, 1999.
- International Federation of Clinical Chemistry (IFCC). Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values (EPTRV). IFCC Document (1982) stage 2, draft 2, 1983-10-07 with a proposal for an IFCC recommendation. The theory of reference values. Part 2. Selection of individuals for the production of reference values. *Clin Chim Acta* 139(2): 205F-213F, 1984.
- International Federation of Clinical Chemistry, Scientific Committee, Clinical Section. Expert Panel on Theory of Reference Values (EPTRV). The theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clin Chim Acta* 137(1): 97F-114F, 1984.
- International Federation of Clinical Chemistry, Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values (EPTRV). Part 6. Presentation of observed values related to reference values. *Clin Chim Acta* 127(3): 441F-448F, 1983.
- Solberg HE: Statistical treatment of reference values in laboratory medicine: testing goodness-of-fit of an observed distribution to the Gaussian distribution. *Scand J Clin Lab Invest Suppl.* 46s 184: 125-132, 1986.
- Solberg HE: REFVAL. The programs are available upon request to H.E. Solberg, Department Clin Chem, Rikshospitalet, N-0027, Oslo 1, Norway, 1983.
- Solberg HE: The IFCC recommendation on estimation of reference intervals. The Refval Program. *Clin Chem Lab Med* 42(7): 710-714, 2004.
- Daele JJ: Chronic sinusitis in children. *Acta Otorhinolaryngol Belg* 51(4): 285-304, 1997.
- Polmar SH, Waldmann TA and Terry WD: The relationship of IgA and IgE deficiency. *Birth Defects Orig Artic Ser* 11(1): 147-150, 1975.
- Berg T and Johansson SGO: Immunoglobulin levels during childhood, with special regard to IgE. *Acta Paediatr Scand* Sep 58(5): 513-524, 1969.
- Pilette C, Durham SR, Vaerman JP and Sibille Y: Mucosal immunity in asthma and chronic obstructive pulmonary disease: a role for immunoglobulin A? *Proc Am Thorac Soc* 1(2): 125-135, 2004.
- Lødrup Carlsen KC, Söderström L, Mowinckel P, Håland G, Pettersen M, Munthe Kaas MC, Devulapalli CS, Buchmann M, Ahlstedt S and Carlsen KH: Asthma prediction in school children; the value of combined IgE-antibodies and obstructive airways disease severity score. *Allergy* 65(9): 1134-1140, 2010.
- Halvorsen R, Jenner A, Hagelin EM and Borres MP: Phadiatop infant in the diagnosis of atopy in children with allergy-like symptoms. *Int J Pediatr* 2009: 460737.
- Garcia-Marcos L, Sanchez-Solis M, Martinez-Torres AE, Lucas Moreno JM and Hernando Sastre V: Phadiatop compared to skin-prick test as a tool for diagnosing atopy in epidemiological studies in schoolchildren. *Pediatr Allergy Immunol* May 18(3): 240-244, 2007.

Received March 5, 2011

Revised May 10, 2011

Accepted May 12, 2011