

Short communication

Imbalanced switch of the IGHG (immunoglobulin constant heavy G chain) Gm(bfn) genes in atopic childhood asthma

Background: The IGHG genes on chromosome 14q32, 5'μ δ γ3 γ1α1 γ2 γ4 ε α2 3', as studied by Gm allotypes, are involved in the inheritance of atopy. The 5'μ δ b f α1 n γ4 ε α2 3', Gm(bfn) haplotype of the genetic B1-cell variant has been found to be associated with the atopic phenotype of children with bronchial asthma.

Methods: An indirect competitive enzyme-linked immunosorbent assay for quantitation in serum of the alternative serum Gm allotypes from the γ3-, γ1-, and γ2 loci and radial immunodiffusion for quantitation of IgG subclasses were used. Children with the genetic B1-cell variants B1/B1 (= Gm[bfn/bfn]), B1/B2 (= Gm[bfn/bf-n]), and B1/B4 (= Gm[bfn/ga-n]) and bronchial asthma were investigated and compared to healthy children of the same age and B-cell type.

Results: The three groups with B1/B1, B1/B2, and B1/B4 cells exhibited increased IgE. In both homozygous and heterozygous B1 or Gm(bfn), the serum G1m(f) levels from γ1 loci were significantly downregulated to 75% of normal, while G2m(n) from γ2 loci were significantly upregulated to about double the normal level. In heterozygous patients with additional B2 or B4 cells, the G2m(-n) levels from γ2 loci were instead downregulated. G1m(a) from γ1 of B4 cells was also downregulated.

Conclusions: Children with atopic bronchial asthma demonstrated an imbalanced class switch in rearrangement of the genes for IgG. The activity of G1m(f) from the γ1 locus was downregulated, but G2m(n) from γ2 was upregulated together with the closely situated ε locus downstream of the IGH genes. Low levels of G1m(f), G1m(a), and G2m(-n) indicated a low pressure of infections. The imbalanced activation of the IGH genes in more hygienic environments might be one explanation of the increased prevalence of atopy in children in recent decades.

V.-A. Oxelius

Department of Pediatrics, University Hospital,
University of Lund, Lund, Sweden

Key words: childhood asthma; genetic B cells; IGHG genes; serum Gm allotype levels.

Dr Vivi-Anne Oxelius
Department of Pediatrics
University Hospital
University of Lund
Se-221 85 Lund
Sweden

Accepted for publication 26 June 2000

The Gm(bfn) haplotype of IGHG genes is related to atopy. The heavy constant chains of immunoglobulin molecules in both IgG and IgE molecules are coded from chromosome 14q32, which contains the genes in the order 5'μ δ γ3 γ1 α1 γ2 γ4 ε α2 3'. The expression from the γ3, γ1, and γ2 loci can easily be detected in serum by two alternative Gm allotypes differing in amino-acid epitopes of the heavy constant γ chains and inherited in a mendelian way. They give rise to four Gm haplotypes and 10 Gm genotypes (1). The four Gm haplotypes characterize four different genetic B-cell variants; namely, Gm(bfn): B1, Gm(bf-n): B2, Gm(ga-n): B3, and Gm(ga-n): B4 (Table 1) (2).

The Gm(bfn) haplotype expressed by B1 cells is associated with increased IgE production in children with bronchial asthma. Our earlier studies on childhood asthma showed that the high-producing ε gene is in linkage disequilibrium with the closely situated G2m(n) of the γ2 gene and is associated with the Gm(bfn)

haplotype expressed by B1 cells (2–7). In Caucasians, G2m(n) is expressed together with G1m(f) of γ1, which also is known to be in linkage disequilibrium with G3m(b) of γ3 (1). Our studies of children with bronchial asthma comparing those with homozygous expression of 5' μ δ b f α n γ4 ε α 3' from B1/B1 cells with children expressing 5' μ δ g a α -n γ4 ε α 3' from B4/B4 cells have revealed two different pathways of immune regulation: different qualities of IgG subclass (Gm) molecules, different IgE levels, and different numbers of specific IgE antibodies, peripheral eosinophils, and peripheral cytotoxic T cells (7).

The alternative Gm allotypes within the same IgG subclass appear as qualitatively different entities, with different immunochemical characteristics, different fractionation rates, different electrophoretic rates, and different half-life times (8, 9). The genetic B-cell variation based on genetic markers for IgG (Gm) (IGHG genes) exhibits different Gm allotypes, different

Table 1. Genetic B cells according to Gm haplotype expression, coded from immunoglobulin constant heavy G chain on chromosome 14q32

Genetic B cells	Genetic code chromosome 14q32	Gm haplotype	Frequency in Caucasian population
	5' μ δ γ 3 γ 1 α 1 γ 2 γ 4 ϵ α 2 3'		
B1	5' μ δ b f α 1 n γ 4 ϵ α 2 3'	Gm(bfn)	44.8%
B2	5' μ δ b f α 1 -n γ 4 ϵ α 2 3'	Gm(bf-n)	22.9%
B3	5' μ δ g a α 1 n γ 4 ϵ α 2 3'	Gm(gan)	0.9%
B4	5' μ δ g a α 1 -n γ 4 ϵ α 2 3'	Gm(ga-n)	31.4%

IgG subclass levels, and different maturation rate during childhood (10, 11). Therefore, it was interesting to examine IgG as Gm allotype levels in children with the most typical and severe atopic bronchial asthma found in the group with homozygous Gm(bfn) haplotypes from B1/B1 cells. They were compared with healthy subjects of the same age and Gm genotype. Patients with atopic bronchial asthma and heterozygous expression of the B1 cell, B1/B2 and B1/B4, were also investigated in the same way.

Material and methods

Patients

Twenty-two children with atopic bronchial asthma, admitted to the outpatient clinic at the Department of Pediatrics, University Hospital of Lund (Sweden), with homozygous expression of Gm(bfn/bfn) and B cells of type B1/B1 were investigated. They were included consecutively, aged 7–17 years, with recurrent obstructive episodes, FEV₁/FVC of <85%, positive reversibility tests after β_2 -agonist challenge, and IgE levels of 518 ± 540 kU/l (Table 2) (22), peripheral blood eosinophils $\times 10^9$ 0.41 ± 0.20 , and positive skin prick tests (SPT) or RAST: 4.3 ± 2.3 (21). Lung-function test, blood sampling, and SPT were performed, as a rule, at the first visit and before treatment with local corticosteroids. No patients were undergoing immunotherapy. All patients and healthy controls were Caucasians. Serum samples were drawn occasionally without

seasonal preference. A healthy control group of 42 individuals of the same age and Gm(bfn/bfn) genotype of B1/B1 cells was chosen from the material of 422 healthy children of different ages and Gm genotypes in the study recently published (11). Two control groups of atopic bronchial asthma with heterozygous expression of B1 cells and Gm(bfn) haplotypes, together with increased IgE, were also investigated (Table 2); 20 with B1/B2 cells (Gm[bfn/bf-n]) and 16 with B1/B4 cells (Gm[bfn/ga-n]). Healthy children of the same age, B cells, and Gm genotypes as the control groups were investigated; 47 with B1/B2 cells and 65 with B1/B4 cells. Three divergent Gm genotypes among our asthmatic children with B2/B2, B4/B4, and B2/B4 cells, all with homozygous G2m(-n) on the γ 2 locus, did not show increased IgE and were excluded.

Gm allotype levels

A competitive ELISA for measuring the amounts of serum G1m(f)-, G1m(a)-, G2m(n)-, and G3m(b) allotypes was used (12), with the following monoclonal antibodies: anti-G1m(f) clone 5F10, anti-G1m(a) clone 5E7, anti-G3m(b1/u) clone 12D9 (Janssen Biochimica, Beerse, Belgium), and anti-G2m(n) clone SH21 (Sigma, St Louis, MO, USA). Purified myeloma proteins of the Gm allotypes G1m(f), G1m(a), G2m(n), and G3m(b) were used to coat the plates. Determination of homozygosity and heterozygosity for the G2m(n) allotype was done by monoclonal precipitating antibody (13). The IgG subclass contents of a normal serum pool from more than 500 blood donors were standardized against a reference WHO serum 67/97, with IgG1 containing, in proportion, G1m(a)/G1m(f) 30/70, IgG2: G2m(n)/G2m(-n) 45/55, and IgG3: G3m(g)/G3m(b) 30/70 (values are given as g/l according to the WHO reference serum 67/97).

Statistical evaluation

Statistical analyses were performed with the *z*-test.

Table 2. Serum levels of alternative Gm allotypes and total IgG3, IgG1, and IgG2, together with IgG4 and IgE (in order as they appear as genes on chromosome 14q32), in genetic B1-cell variants. Investigation of children with atopic bronchial asthma (A) and age-matched healthy children (H)

Genetic B cells	Gm genotype	Number	IgG3 G3m allotypes			IgG1 G1m allotypes			IgG2 G2m allotypes			IgG4 g/l	IgE kU/l
			G3m (b) g/l	G3m (g) g/l	IgG3 total g/l	G1m (f) g/l	G1m (a) g/l	IgG1 total g/l	G2m (n) g/l	G2m (-n) g/l	IgG2 total g/l		
B1/B1	Gm(bfn/bfn)	A(22)	0.63 ± 0.25		0.63 ± 0.25	6.67 ± 1.71^a		6.67 ± 1.71^a	2.99 ± 1.03^a		2.99 ± 1.03^a	0.62 ± 0.75	518 ± 540
		H(42)	0.61 ± 0.28		0.61 ± 0.28	9.10 ± 2.60^a		9.10 ± 2.60^a	1.57 ± 0.84^a		1.57 ± 0.84^a	0.58 ± 0.39	
B1/B2	Gm(bfn/bf-n)	A(20)	0.70 ± 0.19		0.70 ± 0.19	6.74 ± 1.11^a		6.74 ± 1.11^a	1.51 ± 0.65^c	1.04 ± 0.65^a	2.53 ± 0.78	0.52 ± 0.35	191 ± 294
		H(47)	0.68 ± 0.27		0.68 ± 0.27	8.78 ± 3.03^a		8.78 ± 3.03^a	1.15 ± 0.63^c	1.74 ± 0.82^a	2.89 ± 1.27	0.63 ± 0.51	
B1/B4	Gm(bfn/ga-n)	A(16)	0.33 ± 0.17	0.20 ± 0.10	0.52 ± 0.22	3.51 ± 0.74^a	2.33 ± 0.91	6.62 ± 1.74^a	1.21 ± 0.52^d	1.25 ± 0.61^b	2.43 ± 0.94	0.42 ± 0.32	461 ± 755
		H(65)	0.31 ± 0.14	0.34 ± 0.18	0.65 ± 0.27	5.23 ± 2.06^a	3.85 ± 1.20	8.46 ± 1.70^a	0.96 ± 0.48^d	1.69 ± 0.80^b	2.66 ± 1.06	0.60 ± 0.54	

^a $P < 0.001$; ^b $P < 0.02$; ^c $P < 0.05$; ^d $P < 0.10$.

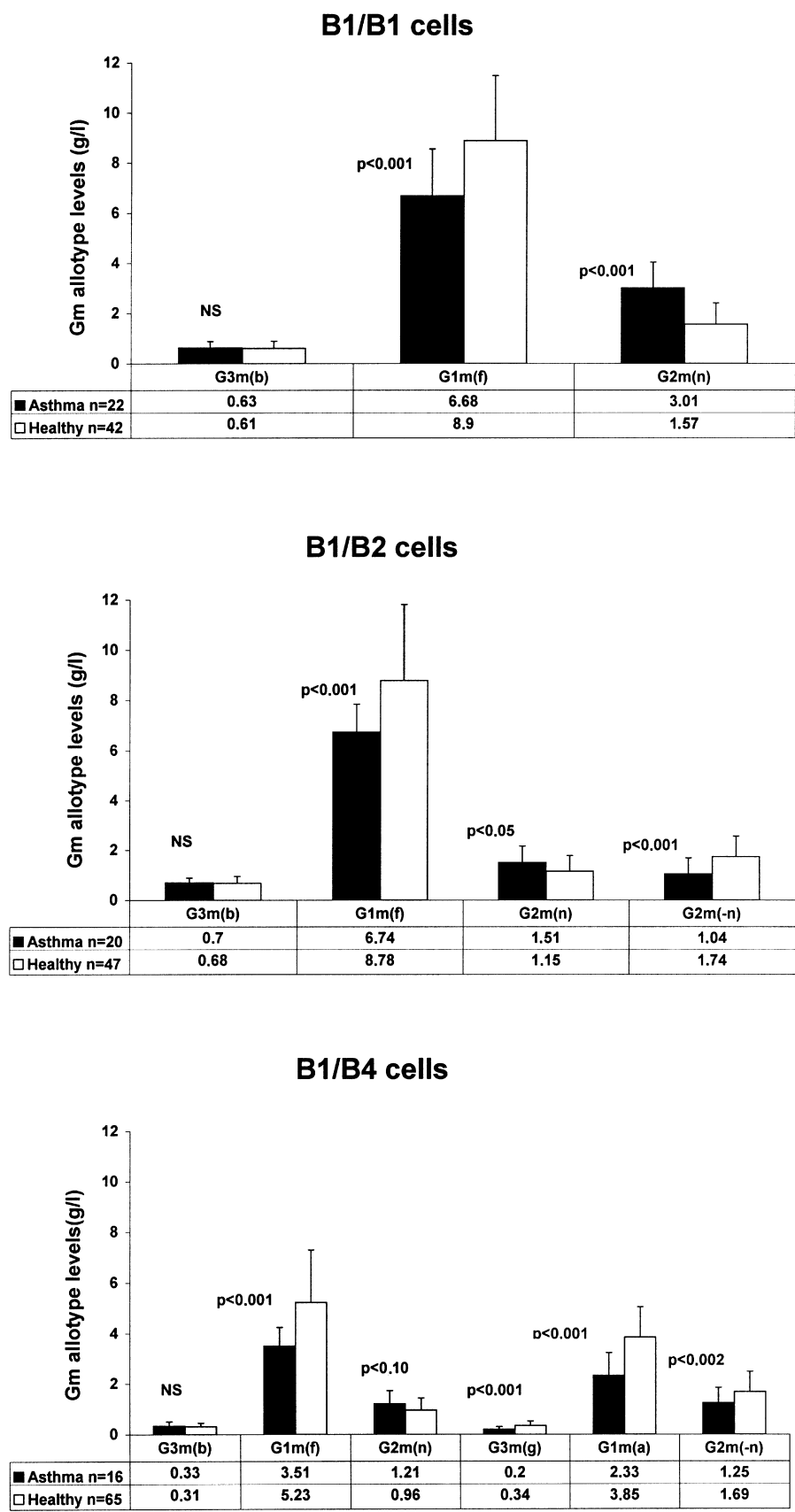


Figure 1. Genetic B1/B1, B1/B2, and B1/B4 cells demonstrating different Gm allotypes and Gm allotype levels in 7–14-year-old children with atopic bronchial asthma. They were compared to healthy children of same age and B-cell type.

Results

Gm allotypes of IgG1: G1m(f) and G1m(a)

G1m(f). The serum levels of G1m(f) (IgG1 antibodies) were significantly low ($P < 0.001$) in children with atopic bronchial asthma and increased IgE. This was found both in patients with B1/B1 and B1/B2 cells with homozygous G1m(f,f) on the $\gamma 1$ locus, and in patients with B1/B4 cells with heterozygous G1m(f,a) on the $\gamma 1$ locus (Table 2). The mean G1m(f) values were about 67–75% of the values found in the healthy groups (Fig. 1).

G1m(a). In patients with B1/B4 cells with G1m(f,a) on the $\gamma 1$ locus, the G1m(a) levels were also significantly decreased ($P < 0.001$). The $\gamma 1$ locus was downregulated in all three Gm genotypes investigated.

Gm allotypes of IgG2: G2m(n) and G2m(-n)

G2m(n). Serum G2m(n) levels (IgG2 antibodies) were instead significantly increased in patients with B1/B1 cells with homozygous G2m(n,n) on the $\gamma 2$ locus and in patients with B1/B2 heterozygous G2m(n,-n) on the $\gamma 2$ locus with the haplotypes Gm(bfn/bf-n) (Table 2, Fig. 1). In patients with B1/B4 cells with G2m(n,-n) on the $\gamma 2$ locus with the haplotypes Gm(bfn/ga-n), the G2m(n) level was increased, but not significantly so ($P < 0.10$).

G2m(-n). Serum G2m(-n) levels in patients with B1/B2 and B1/B4 with the additional Gm(bf-n) haplotype and the additional Gm(ga-n) haplotype, both genetic groups with heterozygous G2m(n,-n) allotypes on the $\gamma 2$ locus, were significantly low compared to healthy subjects. G2m(n) from the $\gamma 2$ locus in asthmatic children was upregulated, but G2m(-n) from the same $\gamma 2$ locus was instead downregulated compared to healthy children.

Gm allotypes of IgG3: G3m(b) and G3m(g)

G3m(b). G3m(b) levels (IgG3 antibodies) did not deviate from those of healthy subjects (Table 2, Fig. 1).

G3m(g). G3m(g) levels from patients with genetic B1/B4 cells or the Gm(bfn/ga-n) genotype were significantly low in asthmatic children compared to healthy children.

Discussion

An imbalanced activation in rearrangement of IGHG genes was seen in children with atopic bronchial asthma and genetic B1 cells (Gm[bfn]) in homozygous or heterozygous states. The activity of G1m(f) antibodies from the $\gamma 1$ locus was downregulated, while G2m(n)

from the $\gamma 2$ locus was upregulated. This was most pronounced in patients with genetic B1/B1 and B1/B2 cells but was less pronounced when B1 cells were mixed with B4 cells. In the rearrangement of genes for immunoglobulins, the first step is the heavy-chain rearrangement of the antigen-binding V(variable)-region. The κ (kappa) locus of light chains is then stimulated, followed by the λ (lambda) locus, if necessary. Once the V-region is determined, it remains essentially unaltered and is then recombined with the constant heavy-chain gene. The class switching is essential for both rearrangement of genes for the antibodies and the function of antibodies.

Fewer G1m(f) antibodies might have been the result of less exposure to infections, as IgG1 antibodies are normally produced in the defense against bacterial and viral infections. Healthy individuals with B1/B1 cells or the Gm(bfn/bfn) genotype have actually the ability to produce the highest levels of specific antibodies, both IgG1 and IgG2 antibodies, against encapsulated bacteria in severe bacterial infections and after vaccination (14–16). The specific antibody response to pneumococcal vaccination has been shown to be fourfold higher in G2m(n) individuals. During childhood, the developmental rate of G1m(f) has been found to be increased over G1m(a) (11).

Downstream the IGH gene, the G2m(n) of the $\gamma 2$ locus, was instead more activated than normal, producing about double the amount of G2m(n) of healthy subjects. Normally, the developmental rate of G2m(n) in healthy children is known to be the most retarded with slowly increasing levels during childhood. G2m(n) does not reach adult levels until late in adolescence (11). On the contrary, children with atopic bronchial asthma showed significantly increased G2m(n) levels, often together with measurable IgG4 levels. The heavy-chain gene loci for both IgG2 and IgG4 are situated downstream of the IGH genes just in front of the heavy-chain locus for IgE on chromosome 14q32. Perhaps the genetic material not occupied with IgG1 antibodies to infectious agents was instead utilized for the $\gamma 2$ locus, the $\gamma 4$ locus, and the ϵ locus. Such an additional involvement of other IGH gene loci can be recognized in IgA-deficient patients, in whom serum levels of IgG3 and IgG1, for which the $\gamma 3$ and $\gamma 1$ loci situated upstream of the $\alpha 1$ locus, are increased with amounts comparable to the amounts missing from the IgA locus. In IgA deficiency, the G2m(n) is partly excluded downstream of a silent $\alpha 1$ locus (17). In B1/B1 cells of children with atopic bronchial asthma, there seems to be a compensatory increase of G2m(n) from the $\gamma 2$ locus comparable to the amount of G1m(f) missing from the $\gamma 1$ locus. However, such a compensation was not seen for G2m(-n) on the $\gamma 2$ locus from B4 cells or the Gm(ga-n) allele expressing low levels of G3m(g) and G1m(a) in addition.

The selection of heavy constant chains for the immunoglobulin molecule from the B cell is usually started upstream of the IGH gene, with a primary requirement for IgM and IgG3 but also IgG1 antibodies in the defense against infectious agents. Utilization of the IGH loci downstream is an effect of a more mature antibody response seen in adulthood or as an effect of chronic stimulation.

The activation of the immune system by relatively small amounts of allergens presented on the mucous membranes results in increased specific IgE antibody production in genetically prone patients, mainly those with B1 cells. Children with genetic B1/B1 cells (homozygous Gm(bfn/bfn)) have the most severe atopic bronchial asthma, expressing the G3m(b) from γ 3, G1m(f) from γ 1, and G2m(n) from γ 2, together with increased IgE levels. It seems that individuals with B1 cells are more sensitive to the influence of IL-4, which upregulates IgE. The association of genetic B cells with cytokines must be investigated further.

The G2m(-n) levels from our children with atopic bronchial asthma with heterozygous G2m(n,-n) showed significantly low levels, compared to healthy children, indicating a low randomized activity of the accompanying allele. This further supports the hypothesis that there was no need for G2m(-n) antibodies for viral or bacterial infections because of an infection-free environment.

Bronchial asthma in children is a heterogeneous disease but with bronchial inflammation in common. Another childhood asthma group with characteristics of nonatopic bronchial asthma has been investigated earlier (7) with homozygous G2m(-n,-n) from B4/B4 cells. Patients with B4/B4 cells exhibit symptoms of recurrent upper and lower respiratory infections with low levels of both G1m(a) as IgG1 antibodies and G2m(-n) as IgG2 antibodies, together with low IgE levels. The low level of G2m(-n) found in patients was thought to derive from an inability to destroy bacterial

or viral invaders, in association with a nonatopic pathway of immune regulation (7). Several studies have found G2m(-n) to be a possible marker of nonatopic asthma (3–7).

Immunodeficiency states favor certain Gm genotypes with homozygous expression of G2m(-n) on the γ 2 locus as in nonatopic bronchial asthma. Immunodeficiencies almost always involve chronic lung disease. IgG2 deficiencies and common variable immunodeficiency are associated with the homozygous Gm(bf-n)(bf-n) genotype of B2/B2 cells, and IgG3 deficiency with the homozygous Gm(ga-n)(ga-n) genotype of B4/B4 cells. In IgG3 deficiency, the most common symptom was chronic obstructive lung disease (18).

Children with atopic bronchial asthma exhibit particular qualities of IgG molecules, namely, G3m(b), G1m(f), and G2m(n) of B1 cells, and imbalanced amounts in serum, compared to healthy children. We suggest that the genetic events in individuals with the IGH gene 5' μ δ b f α 1 n γ 4 ϵ α 2 3' on chromosome 14q32, in an environment relatively free from infections, result in an imbalanced switch of IgG molecules and activation of the ϵ locus, resulting, in turn, in increased IgE levels. These genetic events may explain the increased prevalence of atopy in children during recent decades in developed countries. These results indicate the need to include the IGHG genes in further research on atopy and various other allergic manifestations.

Acknowledgments

This study was supported by grants from the Swedish Medical Research Council (K97-19X-11625-02B), the medical Faculty of the University of Lund and the University Hospital of Lund, the Swedish Asthma and Allergy Association, and the Vardal Foundation.

References

- GRUBB R. Human immunoglobulin allotypes and mendelian polymorphism of the human immunoglobulin genes. In: OSS CJ, REGENMORTEL MHV, editors. *Immunochemistry*. New York: Dekker, 1994:47–68.
- OXELIUS V-A. Genetic B cell variation based on immunoglobulin heavy G chain (Gm) genes. *Scand J Immunol* 1999;49:345–346.
- OXELIUS V-A. Correlation between atopy and Gm allotypes. *Int Arch Allergy Appl Immunol* 1990;91:54–57.
- OXELIUS V-A. Gm allotype genes and gene dosage affecting both IgG subclass and IgE levels in atopic patients. *Int Arch Allergy Appl Immunol* 1990;91:58–61.
- OXELIUS V-A, HULTQUIST C, HUSBY S. Gm allotypes as indicators of non-atopic and atopic bronchial asthma. *Int Arch Allergy Appl Immunol* 1993;101:66–71.
- OXELIUS V-A, SJÖSTEDT L, WILLERS S, LÖW B. Development of allergy to laboratory animals is associated with particular Gm and HLA genes. *Int Arch Allergy Immunol* 1996;110:73–78.
- OXELIUS V-A, CARLSSON A-M, AURIVILLIUS M. Alternative G1m, G2m and G3m allotypes of IGHG genes correlate with atopic and nonatopic pathways of immune regulation in children with bronchial asthma. *Int Arch Allergy Immunol* 1998;115:215–219.

8. OXELIUS V-A, EIBL MM. Different Gm allotype levels in human intravenous immunoglobulin (IVIG) preparations; survival of foreign Gm allotypes in immunodeficient patients. *Clin Exp Immunol* 1996;**106**:203–207.
9. OXELIUS V-A. Preparation of IgG subclass allotypes from polyclonal IgG. *Scand J Immunol* 1999;**49**:395–398.
10. OXELIUS V-A. Serum IgG and IgG subclass contents in different Gm phenotypes. *Scand J Immunol* 1993;**37**:149–153.
11. OXELIUS V-A, AURIVILLIUS M, CARLSSON A-M, MUSIL K. Serum Gm allotype development during childhood. *Scand J Immunol* 1999;**50**:440–446.
12. OXELIUS V-A, CARLSSON A-M. Quantitation of Gm allotypes. *Scand J Immunol* 1993;**37**:143–148.
13. RAUTONEN N, SEPPÄLÄ I, HALLBERG T, GRUBB R, MÄKÄLÄ O. Determination of homozygosity and heterozygosity for the G2m(n) allotype by monoclonal precipitating antibody. *Exp Clin Immunogenet* 1989;**6**:31–37.
14. PANDEY J, FUDENBERG HH, VIRELLA G, et al. Association between immunoglobulin allotypes and immune response to *Haemophilus influenzae* and meningococcus polysaccharides. *Lancet* 1979;**i**:190–192.
15. AMBROSINO D, SCHIFFMAN G, GOTSCHLICH E, et al. Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. *J Clin Invest* 1985;**75**:1935–1942.
16. GRANHOFF DM, SUAREZ BK, PANDEY JP, SHACKLEFORD PG. Genes associated with the G2m(23) immunoglobulin allotype regulate the IgG subclass responses to *Haemophilus influenzae* type b polysaccharide vaccine. *J Infect Dis* 1988;**157**:1142–1149.
17. OXELIUS V-A. Lack of the G2m(n) allotype in IgG subclass deficiency: in IgG2 deficiency together with lack of G1m(a) and G3m(g) and in IgG3 deficiency together with lack of G1m(f) and G3m(b). *Scand J Immunol* 1990;**31**:243–247.
18. OXELIUS V-A, HANSON L-Å, BJÖRKANDER J, HAMMARSTRÖM L, SJÖHOLM A. IgG3 deficiency: Common in obstructive lung disease. *Monogr Allergy* 1986;**20**:106–115.