Clin. exp. Immunol. (1992) **90**, 199–203

Herpesvirus serology, aberrant specific immunoglobulin G2 and G3 subclass patterns and Gm allotypes in individuals with low levels of IgG3

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(Accepted for publication 15 July 1992)

SUMMARY

One objective of this study was to determine whether IgG3-deficient individuals have an increased frequency of reactivated herpesvirus infections. Serum titres to Epstein–Barr virus (EBV) and human herpesvirus-6 were examined in 10 healthy and in 10 symptomatic persons with serum IgG3 $< 0{\cdot}1$ g/l. Atypical titres were found in 16% of the IgG3-deficient individuals. Reactivations of these viruses thus do not seem common in IgG3 deficiency. Antigen-specific IgG responses were also determined. A lowered frequency of IgG3 to an EBV-derived peptide was found only in symptomatic, IgG3-deficient individuals. Levels of IgG2 to a bacterial polysaccharide were lowered in the same group, despite normal serum levels of total IgG2. A functional IgG2 deficiency may contribute to symptoms in IgG3 deficiency. The G3(g) allotype, known to be associated with low total IgG3, dominated in IgG3-deficient persons (13/17) independently of presence or absence of symptoms. A linkage of G3(g) to the G2(n) negative allotype, associated with low IgG2, was equally common irrespective of symptoms. G3(g) and absence of G2(n) seem to be one prerequisite for most of IgG3 deficiency combined with low specific IgG2.

Keywords IgG3 deficiency Epstein–Barr virus HHV-6 pneumococcus IgG subclasses Gm allotypes

INTRODUCTION

IgG3 deficiency has been reported in patients with recurrent upper respiratory tract infections, recurrent fever and fatigue [1]. Human antibodies to viral antigens are predominantly of the IgG1 and IgG3 subclasses [2,3]. While anti-viral IgG1 dominates quantitatively, IgG3 is a more efficient mediator of viral neutralization and antibody-dependent cellular cytotoxicity (ADCC; [4,5]). Lack of specific IgG3 may thus in theory impair the anti-viral defence, and result in prolonged or severe viral infections. In order to examine this, IgG titres to two latent herpes viruses, Epstein–Barr virus (EBV) and human herpesvirus-6 (HHV-6) antigens, were compared in healthy and symptomatic individuals with low serum IgG3 of $< 0{\cdot}1$ g/l and in normoglobulinaemic controls. Elevated EBV and HHV-6 titres in deficient individuals would indicate that IgG3 deficiency could result in increased replication of latent viruses.

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The symptoms reported in IgG3 deficiency [1] bear similarities to those characteristic of the chronic fatigue syndrome [6]. Various IgG subclass deficiencies including IgG3 deficiency have been found in patients with chronic fatigue syndrome, and many such patients also have high IgG titres to antigens of EBV and HHV-6 [7]. It was thus of clinical interest to choose these two herpes viruses for study.

The lower normal value of serum IgG3 has varied in different studies [8–10], and a relevant normal limit value for total IgG3 in serum is not yet firmly established. Furthermore, preliminary results indicate that there is no complete correlation between levels of the total serum IgG3 and specific IgG3 reactivity [11]. The second aim of this study was to investigate whether patients with very low total IgG3 levels of $< 0{\cdot}1$ g/l generally have reduced levels of antigen-specific IgG3, or other deficiencies in specific IgG subclass reactivities. The IgG subclass distribution of specific antibodies reactive with one protein and one polysaccharide antigen was compared in IgG3-deficient and normoglobulinaemic individuals. Since low IgG subclass levels may in fact be the result of normal, allotypic

Table 1. Sex, age and symptoms of patients with IgG3 deficiency and their Epstein–Barr virus capsid antigen (VCA), early antigen complex (EA), p107 and HHV-6 IgG titres. OD_{490} values for IgG3 to p107 and OD values for IgG2 to PPS6A are also included

Patient no.	Sex	Age (years)	Symptoms	VCA G (titres)	EA G (titres)	p107 G (titres)	HHV-6 G (titres)	p107 G3 OD_{490}	PPS6A G2 OD_{410}
1	M	20	Acute idiopathic thrombocytopaenic purpura	≥1280	≥80	<100	≥1280	<0·2	2·35
2	M	46	Ulcerative colitis, recurrent fever	320	≤20	>1000	20	<0·2	2·12
3	F	51	Chronic fatigue syndrome	≥1280	>80	>1000	≥1280	<0·2	1·88
4	F	59	Recurrent bronchitis	320	≥80	>1000	80	<0·2	1·19
5	F	44	Recurrent upper respiratory tract infections	320	≤20	>1000	20	0·90	1·16
6	F	37	Food allergy	<20	<20	<100	80	<0·2	1·08
7	F	66	Recurrent purulent skin and respiratory tract infections	160	≤20	>1000	80	0·24	0·97
8	F	57	Rheumatoid arthritis	≥1280	≤20	>1000	20	0·26	0·82
9	F	33	Acne, recurrent throat infections	80	<20	>1000	320	0·43	0·69
10	M	42	Recurrent upper respiratory infections	80	≤20	>1000	80	<0·2	0·11

variants G3(g) [12–14], we related our findings to the Gm haplotypes of the patients.

SUBJECTS AND METHODS

Serum samples

One serum sample from each of 10 symptomatic individuals (mean age 44 years; seven women, three men), with total serum IgG3 levels ranging from 0·01 to 0·05 g/*l* as measured by radial immunodiffusion [15] was studied. Their serum levels of the other IgG subclasses were all within the normal range (IgG1, 7–14·2, mean 10·4; IgG2, 1·6–4·6, mean 3·3; IgG4, 0·1–1·02, mean 0·4 g/*l*) [10]. The patients had been referred for examination of IgG subclasses owing to various symptoms of unexplained etiology, of which recurrent respiratory tract infections dominated (Table 1). Two healthy controls for each patient, matched for age and sex, and with normal IgG subclass levels [10] were examined in parallel. Ten subjectively healthy blood donors, in whom a total serum level of IgG3 of ≤0·1 g/*l* was discovered during a study of IgG3 subclass levels in healthy young men, were also investigated.

Assays for IgG and IgM to EBV and HHV-6

IgG and IgM to the EB virus capsid antigen (VCA) and to the EBV early antigen complex (EA) were measured with indirect immunofluorescence assays (IFA) according to previously described methods [16]. EBV VCA IgG titres of ≥1280 together with EA titres of ≥80 occur in <2·5% of healthy individuals, and were regarded as high titres [16]. Presence of antibodies to the EBV nuclear antigen (EBNA) was evaluated with ELISA. As antigen, p107, a peptide representing part of the EBV nuclear antigen 1 (EBNA1) [17,18] was used in its dimeric form (synthesized by Ferring AB, Malmö, Sweden). The ELISA was performed entirely according to previously published methods [18]. In EBV VCA^+ persons, a low p107 IgG titre of <1000 in combination with a p107 IgG/IgM optical density (OD) ratio of <1 are characteristic of recent, primary EBV infection. When p107 IgG is absent, an examination for EBNA antibodies with the anticomplement immunofluorescence method (ACIF) is necessary to verify that the patient lacks EBNA antibodies [18]. A p107 titre of <100 and an EBNA ACIF titre of <2 occurs in <2·5% of healthy EBV^+ individuals, and were regarded as atypical titres in this study.

HHV-6 IgG and IgM titres were also measured with IFA [19,20]. Detectable IgM and/or IgG titres of ≥640 are found in <2·5% of healthy individuals, and were regarded as high titres. HHV-6 IgM and high titres of HHV-6 IgG may be found during EBV and cytomegalovirus infections [19–21].

Assays for IgG subclasses

ELISAs for IgG subclasses reactive with p107 were performed as previously described for other viral antigens [22]. Microplates coated with 0·5 μg peptide/well were incubated with serum dilutions (10^{-1}–10^{-3}) in quadruplicate. After washing, appropriate dilutions [22] of MoAbs to the four subclasses were added (NL16, HP 6014, ZG4 and RJ4; Unipath, Bedford, UK). Peroxidase-labelled rabbit-antimouse immunoglobulin (Dakopatts) was used as the conjugate, and orthophenylenediamine as the substrate. The cut off for a positive reaction was initially determined by assaying 10 EBV^- samples. The mean OD at 490 ± 3 s.d. (around 0·2) was used as the cut-off value for positive reaction. The 10^{-2} dilutions of the sera gave the optimal positive/negative ratio, and were used for evaluation of results.

IgG1 and IgG2 to purified polysaccharide antigen serotype 6A from *Pneumococcus pneumoniae* (PPS6A; MSD Rahway, NJ) was determined according to published procedures [23]. Serum samples were examined at a dilution of 10^{-2}. Clones NL16 and GOM1 (Unipath) were used for the detection of IgG1 and IgG2, respectively, and alkaline phosphatase-labelled rabbit-antimouse immunoglobulin (Dakopatts) as conjugate. ODs at 410 nm were used for presentation of results. All serum samples studied for specific IgG subclasses were examined in the same assay. Intraassay OD variation was <15%.

Table 2. Number (%) of p107 IgG seropositives with measurable IgG1–4 antibodies reactive with p107

	p107 IgG1	p107 IgG2	p107 IgG3	p107 IgG4
Healthy donors	20/20 (100)	10/20 (50)	18/20 (90)	5/20 (25)
IgG3-deficient controls	8/8 (100)	0/8 (0)	6/8 (75)	3/8 (38)
IgG3-deficient patients	9/9 (100)	1/9 (11)	4/9 (44)	3/9 (33)

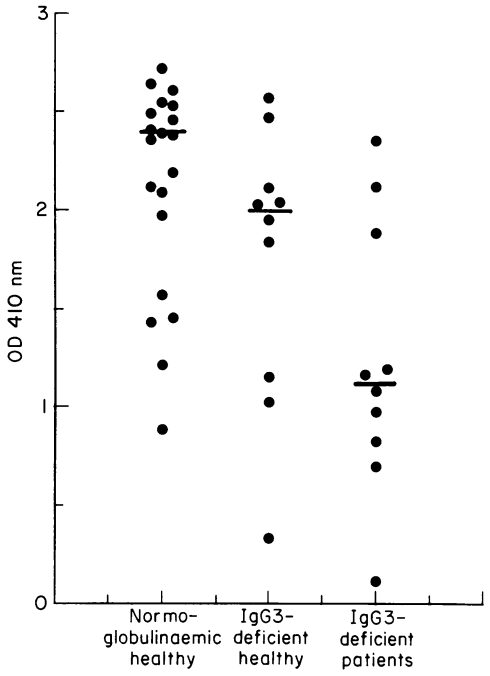

Fig. 1. IgG2 OD at 410 nm to the PPS6A antigens in the three groups. Median values are shown by the horizontal bar.

Examinations of Gm allotypes

Gm allotypes G1(a, ax, f), G2(n), G3(b, g) were determined in 16 out of 20 of the IgG3-deficient individuals. In one further patient G2(n) and G3(g) were examined. The examinations were performed according to previously published methods [24].

RESULTS

Total IgG and IgM to the EBV and HHV-6 antigens

All 20 of normoglobulinaemic controls and nine out of 10 of both the symptomatic and asymptomatic, IgG3-deficient persons, had IgG antibodies to EBV VCA, as a sign of EBV seropositivity. All of the seropositive IgG3-deficient controls had EBV VCA and EA titres within the normal range. In seven out of nine of the symptomatic IgG3-deficient patients EBV VCA and EA IgG titres were normal. Patients 1 and 3 had elevated EBV, VCA and EA IgG titres (Table 1). Patient 1 also expressed IgM to VCA and lacked EBNA antibodies by ACIF. This serology is characteristic of recent, primary EBV infection. The patient was ill with thrombocytopaenic purpura shortly before the serum was taken. In a sample drawn 2 years after his purpura, he had seroconverted to EBNA. IgM to VCA and IgG to EA were absent and IgG to VCA had fallen to 320. His serum IgG3 had then increased to 0·3 g/*l*. It is probable that the EBV titres found in this study were the result of a primary EBV infection preceding the purpura.

IgG to p107 was present in all IgG VCA$^+$, normoglobulinaemic patients, and in all symptomatic IgG3-deficient patients, except for patient 1, who had a probable recent primary EBV infection. In one out of nine EBV$^+$, IgG3-deficient healthy persons the p107 IgG titres were < 100 and EBNA antibodies were not detectable by ACIF.

HHV-6 IgG (titre ≥ 20) was detected in 18 out of 20 healthy, normoglobulinaemic persons, in all healthy and in eight out of 10 symptomatic, IgG3-deficient patients. Patients 1 and 3 who also had elevated EBV titres, were the only ones in the examined material with elevated HHV-6 IgG titres.

Distribution of antigen-specific IgG subclass antibodies

All p107$^+$ individuals expressed IgG1 to p107 (Table 2). There were no differences in OD values for IgG1 between the three groups. IgG2 to p107 was detected in 50% of the healthy, normoglobulinaemic individuals, but in only one out of 17 (6%) of the p107$^+$ IgG3-deficient individuals (Table 2; $P < 0{\cdot}01$; Fisher). Seventeen out of 20 normoglobulinaemic persons expressed IgG3 to p107, which was a significantly higher frequency than that found among the symptomatic IgG3-deficient individuals (four out of nine, $P < 0{\cdot}05$; Fisher). However, it was not higher than among healthy IgG3-deficient persons (six out of eight; Table 2). Traces of IgG4 to p107 were found in 30% of the examined samples, and in the same frequency in all groups.

IgG1 and IgG2 antibodies to the PPS6A antigen were found in all examined samples. The median OD of IgG1 to PPS6A was highest in the symptomatic, IgG3-deficient persons, and lowest in the healthy, normoglobulinaemic ones, but the differences were not significant. The ODs for IgG2 were significantly higher in healthy, normoglobulinaemic persons than in symptomatic, IgG3-deficient patients ($P < 0{\cdot}001$, Mann–Whitney; Fig. 1). The ODs in IgG3-deficient, healthy persons were intermediary and did not differ significantly from either of the other groups. All six patients with recurrent respiratory symptoms as their major complaint had an IgG2 OD to PPS6A below 1·2, which was close to the median value (1·12) in the group of symptomatic, IgG3-deficient patients. There was no correlation between the levels of total serum IgG2 and ODs for specific IgG2.

Gm allotype markers and specific IgG subclass reactivity (Table 3)

The finding of low levels of specific IgG2 in IgG3-deficient persons led to the hypothesis that the G3(g) allotype in combination with absence of G2(n) would dominate in these persons. Gm markers were determined in 17 serum samples available. In 13 out of 17 the G3(g) allotype was found, and 12

Table 3. Frequency of G3(g) and G2(n) allotypes in symptomatic and asymptomatic IgG3-deficient individuals. Median (range) of the ODs for anti-PPS6A IgG1 and IgG2 and anti-p107 IgG3 are also given for the different groups

Symptomatic	Allotype	Frequency	PPS6A OD IgG1	PPS6A OD IgG2	p107 OD IgG3
Yes	$g^+ n^+$	0/9	—	—	—
Yes	$g^+ n^-$	6/9	0·3 (0·3)	1·1 (1·1)	0·2 (0·8)
Yes	$g^- n^+$	2/9	0·3; 1·2*	2·4; 2·1*	<0·2; <0·2*
Yes	$g^- n^-$	1/9	0·2	0·4	0·7
No	$g^+ n^+$	1/8	1·7	2	>2
No	$g^+ n^-$	6/8	0·6 (0·8)	2·0 (1·5)	0·6 (1·3)
No	$g^- n^+$	1/8	0·4	2·6	0·5
No	$g^- n^-$	0/8	—	—	—

* Individual values.

out of these 13 were $G2(n)^-$. One further patient lacked both g and n. The frequences of G3(g), $G2(n)^-$ were almost identical in symptomatic and asymptomatic IgG3-deficient individuals. Of the symptomatic patients, the two (nos 1 and 3) with atypical EBV titres and high levels of IgG2 to PPS6 were the only ones who expressed G2(n). In the asymptomatic group the relation between G2(n) and levels of specific IgG2 was less obvious, but the difference in IgG2 to PPS6 between $G2(n)^+$ and $G2(n)^-$ in all IgG3-deficient individuals was still significant ($P<0·05$, Mann–Whitney). The levels of IgG3 to p107 did not correlate significantly to the absence or presence of the G3(g) allotype. Neither could a distinct correlation between G1(a, ax), G1(f) and IgG1 to PPS6 be found.

DISCUSSION

This study was designed in order to assess whether patients with low levels of IgG3 are prone to reactivate latent herpesvirus infections. The serological response to EBV and HHV-6 differed from that found in normoglobulinaemic individuals in only three out of 18 seropositives. Reactivations of these herpesviruses thus do not seem to be a common consequence of IgG deficiency. Two EBV^+, symptomatic, IgG3-deficient patients had both elevated EBV and HHV-6 titres. These two patients were the only symptomatic IgG-deficient individuals who lacked G3(g) allotype and were $G2(n)^+$. One of these patients had EBV serology diagnostic of primary EBV infection with normalization of the EBV titres and the IgG serum level after 2 years. The other patient fulfilled the clinical criteria proposed for chronic fatigue syndrome [6], in which elevated EBV and/or HHV-6 are frequently encountered. The absence of G3(g) and presence of G3(n) in these two patients indicate that their IgG3 deficiency is not genetically linked, and in one of the patients the deficiency was only temporary. In preliminary experiments we have not found IgG3 depression during mononucleosis in five patients followed for 3 years (unpublished results). IgG3 depression does not seem to be a common consequence of active EBV infection. Probably the atypical EBV symptoms and the low IgG levels are both the result of some other, not yet identified, immune abnormality. The relation between serum levels of IgG subclasses and antigen-specific IgG reactivity was also examined. The evaluation of the clinical importance of low total serum IgG3 is complicated by the lack of definition of IgG3 deficiency [8–10]. In this study we examined individuals with serum IgG3 levels of $<0·1$ g/*l*. Measurable specific IgG3 to an EBV encoded peptide, p107, was found also with these low total levels of IgG3, but was significantly less frequent in the group of symptomatic IgG3-deficient individuals as compared with healthy, normoglobulinaemic persons. This may imply that it is the ability of the patients to produce specific IgG subclasses rather than the total amount in serum of any single subclass that has pathogenic importance, as has also been suggested previously [25, 26].

Antiviral IgG2 is seldom found, but it has been reported to some virus-encoded peptides [27]. In the present study a significantly lower frequency of IgG2 to p107 in symptomatic IgG3-deficient persons than in healthy controls was found. The reactivity of IgG2 to a bacterial polysaccharide was also significantly lower in symptomatic, IgG3-deficient individuals than in healthy controls, though they had normal levels of total IgG2 in serum. IgG2 deficiency has been suggested to cause recurrent bacterial infections [28]. All the IgG3-deficient patients reporting recurrent respiratory tract infections as their major complaint in this study had specific IgG2 reactivity to PPS6A below the mean of the group of IgG3-deficient patients. A functional IgG2 deficiency may contribute to some of the symptomatology found in the IgG3-deficient patients. Examination of specific IgG2 seems indicated in patients with symptoms compatible with impaired immune function and low serum IgG3 levels.

The finding of low antigen-specific IgG2 in patients with IgG3 deficiency led us to speculate that the symptomatic individuals would have the G3(g) allotype, common in IgG3 deficiency and the $G2(n)^-$ allotype. The $G2(n)^-$ allotype is linked to G3(g) and to low levels of total IgG2 and/or specific IgG2 [29]. Allotyping was performed on 17 remaining/obtainable serum samples. The G3(g), $G2(n)^-$ allotypes were found in the majority (71%) of all IgG3-deficient individuals, but equally distributed among healthy and symptomatic persons.

The linkage between G3(g) and absence of G2(n) [30] may help to explain the 'functional' IgG2 deficiency found in the IgG3-deficient patients examined in this study but the reason

why some G2(n)$^-$ patients are less efficient than others in producing specific IgG2 still has to be explained. In a minor subgroup, IgG3 deficiency may be temporary, not genetically linked, and occur in connection with, for example, active EBV infection.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council.

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