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Isotype distribution of mucosal IgG-producing cells in patients with various IgG subclass deficiencies

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SUMMARY

The subclass distribution of IgG-producing immunocytes was examined by immunohistochemistry in nasal and rectal mucosa of infection-prone patients with untreated IgG subclass deficiencies. Biopsy specimens from the two sites were obtained in 18 clinically and serologically well-characterized adult subjects; only a nasal or rectal sample was available from nine similar patients. Chronic lung disease was common in the patient groups with selective serum IgG1 deficiency and combined IgG1 and IgG3 deficiency, whereas the other categories of patients had mainly upper airway and other mild infections. Serum IgG2 or IgG3 deficiency was usually expressed also at the cellular level in rectal mucosa, and the proportion of rectal IgG1 cells was significantly correlated with the IgG1 level ($r=0{\cdot}90$, $P<0{\cdot}001$). Likewise, there tended to be a decreased expression of the actual subclass at the cellular level in nasal mucosa of patients with serum IgG1 or IgG2 deficiency. Conversely, the median nasal proportion of IgG3 cells was remarkably unaffected by a deficiency of this subclass in serum and rectal mucosa. Interestingly, these patients rather tended to have raised IgG3 and reduced IgG2 cell proportions in their nasal mucosa, although this apparent local IgG3 compensation was nevertheless strongly correlated with the serum IgG3 level ($r=0{\cdot}87$, $P<0{\cdot}002$). These disparities may reflect different antigenic and mitogenic exposure of the two tissue sites; for example, a persistent protein bombardment of the nasal mucosa that could conceivably override locally a B cell maturation defect. The possible clinical consequences of such variable mucosal expression of IgG subclass deficiencies remain to be studied.

Keywords IgG subclass deficiencies immunoglobulin-producing cells nasal and rectal mucosa mucosal immunity immunodeficiency

INTRODUCTION

Most human secretory tissues normally contain a substantial although varying number of IgM-, IgG-, and IgD-producing cells in addition to the predominating IgA immunocytes (Brandtzaeg *et al.*, 1979). The proportion of IgG cells is small (3–5%) in normal intestinal mucosa (Crabbé, Carbonara & Heremans, 1965; Brandtzaeg *et al.*, 1985) but considerably greater in respiratory mucosa. Thus, clinically normal nasal mucosa shows an IgG cell distribution of about 11% in glandular areas and 49% in the stroma beneath the surface epithelium; a moderate degree of chronic rhinitis will on average raise these proportions to 24% and 68%, respectively (Brandtzaeg, 1985).

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Information has recently become available concerning synthesis of the four IgG subclasses in the human gut (Scott *et al.*, 1986) and the normal distribution of mucosal immunocytes producing them (Bjerke & Brandtzaeg, 1990; Helgeland *et al.*, 1991). Other studies have considered the influence on this distribution of certain gut diseases such as ulcerative and Crohn's colitis (Kett, Rognum & Brandtzaeg, 1987), chronic gastritis (Valnes & Brandtzaeg, 1989) and coeliac disease (Rognum *et al.*, 1989). IgG1 is the major mucosal IgG subclass in health and disease. A notable difference between the intestinal and nasal mucosa is that IgG2 dominates over IgG3 immunocytes in the former, whereas the reverse is often true in the latter (Brandtzaeg *et al.*, 1986).

The primary aim of this investigation was to examine by immunohistochemistry the IgG immunocyte subclass distribution in nasal and rectal mucosa of serologically IgG subclass-deficient patients. The availability of a serologically and clini-

Table 1. Infections and associated diseases in the patients in relation to their serological IgG subclass deficiencies

Clinical features	Distribution of clinical features according to immunodeficiency						
Infections							
Recurrent rhinopharyngitis		+	+ + + + + + + +		+	+	+ +
Recurrent rhinosinusitis		+					
Chronic bronchitis	+ +	+		+	+ + + + +	+	+ +
Conjunctivitis						+	
Cystitis		+					
Osteomyelitis		+					
Unclassified			+				
Associated diseases							
Chronic lung disease	+ +	+ +	+	+	+ + + +		
Diabetes mellitus	+						
Protein-loosing gastropathy						+	
Hypothyreoid disease	+						
Serological IgG subclass deficiency*	IgG1 (2)	IgG2 (4)	IgG3 (10)	IgG1+2 (1)	IgG1+3 (6)	IgG2+3 (2)	IgG1+2+3 (2)

* Number of patients in parentheses.

cally well-characterized patient material at the University of Göteborg (Söderström *et al.*, 1986; Söderström, Söderström & Hanson, 1987) encouraged us to study relations between the expression of IgG subclass deficiency in serum and mucosa, and the associated clinical manifestations. A preliminary study had suggested that the nasal expression of IgG subclass deficiency at the cellular level does not necessarily conform with the isotype defect seen in serum (Brandtzaeg *et al.*, 1986).

MATERIALS AND METHODS

Patients

Twenty-seven serologically and clinically well-characterized IgG subclass-deficient patients (15 women and 12 men; median age 37 years, range 19–73) were included in the study. They were referred to Sahlgren's Hospital, the University of Göteborg, mainly because of recurrent infections of the respiratory tract; in addition, one-third of the patients had chronic lung disease (mainly bronchial asthma) and a few had associated diseases like insulin-dependent diabetes mellitus, protein-loosing gastropathy, and hypothyreoid disease (Table 1). All patients had total serum IgG, IgA and IgM levels within the normal range. They were subjected to thorough clinical investigation, and the IgG subclass deficiency was confirmed in at least two serum samples, obtained 3–6 months apart (not during or immediately after an acute infection). Sixteen of the patients were included in a controlled study of the effect of immunoglobulin prophylaxis (Söderström *et al.*, manuscript in preparation). The mean number of days with infection per year in these patients was 91·2, as determined during a prospective 12-month period with saline injections. None of the patients was given immunoglobulin during a period of at least 6 months before the biopsy was performed. Informed consent was obtained from all patients, and the study was approved by the Ethic's Committee of the University of Göteborg.

Serum immunoglobulin quantifications

All immunoglobulin determinations were performed by single radial immunodiffusion according to Mancini, Carbonara &

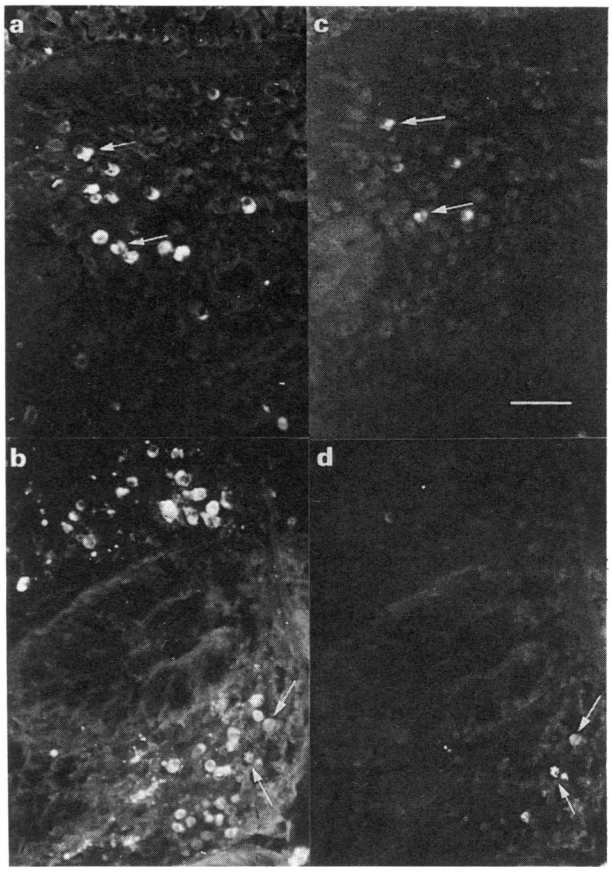

Fig. 1. Two-colour immunofluorescence staining for IgG (a,b, rhodamine) and IgG3 (c,d, fluorescein) in same field. (a,c) Section of nasal mucosa with superficial moderate inflammation; (b,d) section of normal rectal mucosa. Both specimens were obtained from the same patient with serological IgG3 deficiency. (c) In nasal mucosa there was a raised proportion of IgG3-producing cells (21%), whereas (d) rectal mucosal IgG3 cells were reduced in harmony with the serological IgG3 subclass defect. Examples of identical cells in the two panels are arrowed. Bar 40 μm.

Table 2. Biopsy material categorized according to histological findings and serological IgG subclass deficiencies of the patients

Biopsy site and histology*	Distribution of histological findings according to immunodeficiency						
Nasal mucosa							
Normal					+++	+	+
Chronic inflammation							
slight		++	+++++	+	++		+
moderate		++	+				
severe	+		+				
Rectal mucosa							
Normal	++	++	+++++++++	+	++++++	+	++
Chronic inflammation							
Serological IgG subclass deficiency	IgG1	IgG2	IgG3	IgG1+2	IgG1+3	IgG2+3	IgG1+2+3

* Based on tissue sections stained with haematoxylin and eosin.

Heremans (1965) using heavy chain-specific rabbit anti-IgG, anti-IgA, and anti-IgM (Dako immunoglobulins, Dakopatts, Glostrup, Denmark) and the Behring standard reference serum (Behringwerke, Marburg-Lahn, FRG). IgG subclass deficiencies were evaluated by quantification of serum IgG subclasses with monoclonal antibodies (HP6007, clone JL512; HP6008, clone GOM1; HP6010, clone Z64; and HP6011, clone RJ4; Unipath, Bedford, UK) in single radial immunodiffusion performed in gel containing 3% polyethylene glycol. A serological subclass deficiency was diagnosed as a concentration below the normal adult population range as determined by Oxelius (1979).

Biopsy specimens

Nasal mucosal specimens were excised from the inferior turbinate bone at least 4 cm from the nasal tip. Rectal mucosal specimens were obtained from the distal third of the rectum, mainly 5–10 cm up on the posterior wall. In 18 patients mucosal specimens were obtained from both sites; nine patients consented only to a nasal or rectal biopsy.

Control specimens of nasal mucosa from six previously reported patients with IgG subclass deficiencies (Brandtzaeg *et al.*, 1986) were evaluated for inter-observer reproducibility.

Immunohistochemistry

The tissue specimens were prior to ethanol fixation and paraffin embedding extracted in cold phosphate-buffered (pH 7·5) isotonic saline for 48 h to avoid interstitial background staining (Brandtzaeg, 1974). Several serial sections from each tissue block were cut at 6 μm, de-waxed and subjected to immunofluorescence staining. The two-colour method used has been described previously, including characteristics of the fluorochrome conjugates (Brandtzaeg *et al.*, 1986), the source of IgG subclass-specific monoclonal antibodies (Jefferis *et al.*, 1985), and other details of the staining procedure (Kett *et al.*, 1987). Briefly, each section was subjected to paired staining for one of the four IgG subclasses and for total IgG. These sections were first incubated with murine monoclonal antibody (ascites 1:800) to IgG1 (HP 6070, clone 2C7), IgG2 (HP 6009, clone GOM2), IgG3 (HP 6047, clone CB1-AH7), or IgG4 (HP 6011, clone RJ4) and subsequently with a mixture of FITC-labelled rabbit anti-mouse IgG and rhodamine B sulphonyl chloride-labelled anti-human IgG. An additional section from each tissue site was subjected to conventional histological evaluation.

Microscopy and cell counting

All sections were evaluated without knowledge of the immunological or clinical status of the actual patient. Fluorescent cells were observed in a Leitz Orthoplan microscope equipped with $\times 25$ and $\times 40$ immersion objectives, an $\times 10$ ocular, and a Ploem-type vertical illuminator with interference filters for selective observation of green or red emission. For each tissue specimen an average of 778 cells (range 200–2517) showing red (class-specific) cytoplasmic staining were examined for concomitant green (subclass-specific) fluorescence. The proportion of cells containing one of the four subclasses was then calculated in relation to the total number of IgG-producing immunocytes detected in the evaluated area of the same section (Fig. 1). Several sections often had to be counted for each subclass in order to obtain a sufficiently high number of IgG cells, especially in the gut mucosa. On average, the proportions of green cells (all four subclasses) added up to slightly below 100% (range 90–103%) for each specimen.

Microscopic evaluation based on staining with haematoxylin and eosin showed normal histology of rectal mucosa in all patients, whereas slight-to-moderate chronic inflammation was often seen in nasal mucosa (Table 2).

IgG immunocyte reference distribution

The normal subclass distribution of IgG-producing cells was determined by paired staining as described above in 10 jejunal control specimens with no histological or immunological abnormalities. Similar data recorded previously in subjects without known IgG subclass deficiency were obtained for nasal mucosa (Brandtzaeg *et al.*, 1986), normal ileal mucosa (Bjerke & Brandtzaeg, 1990) and normal colonic mucosa (Helgeland *et al.*, 1991).

Statistical analysis

Correlations between the proportions of the four IgG subclasses observed at the two mucosal sites and the IgG-subclass levels in

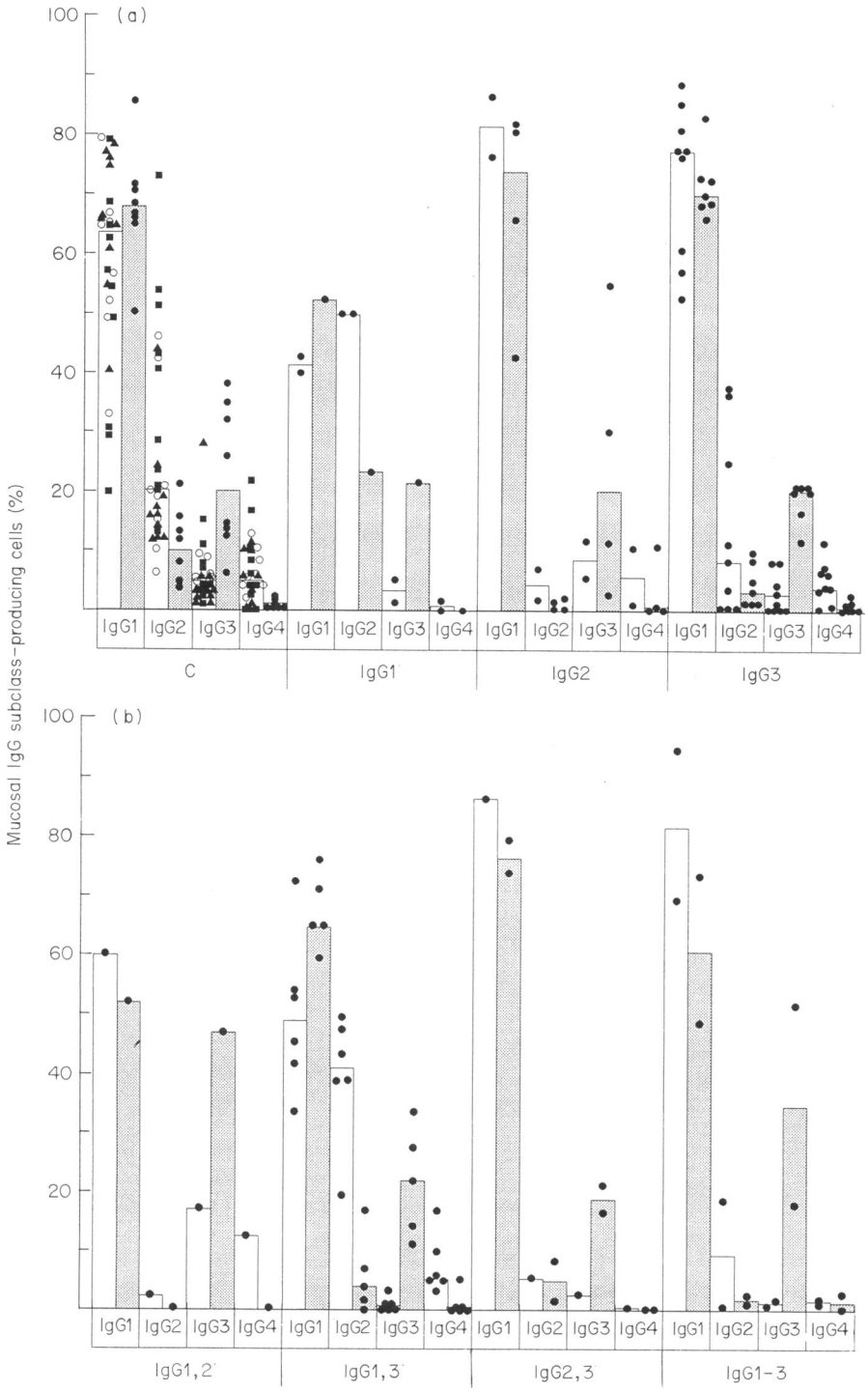

Fig. 2. Median percentage distribution of IgG subclass-producing cells in intestinal (□) and nasal (●) mucosa. (a) Control subjects and patients with single serological IgG subclass deficiencies; (b) patients with combined serological IgG-subclass deficiencies. ▲, jejunum; ○, ileum; ■, colon; ●, rectum.

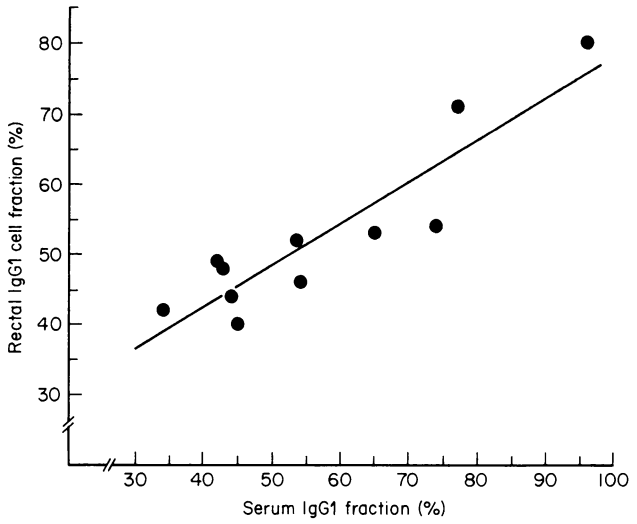

Fig. 3. Regression line for the relation between rectal IgG1 cell fractions and serum IgG1 levels (percentage of total IgG) in eleven patients with single or combined IgG1 deficiency ($r = 0{\cdot}90$, $P < 0{\cdot}001$).

serum at the time of the biopsy, were determined by Pearson's r-test. The inter- and intra-observer reproducibility of fluorescent-cell enumerations were determined by the one-sided sign test.

RESULTS

Chronic lung disease was present in both patients with selective serum IgG1 deficiency and in four of six patients with combined IgG1 and IgG3 deficiency; only two of four patients with selective IgG2 deficiency had this serious disease. The other categories of patients had mostly upper airway or other mild infections (Table 1).

Serum IgG2 or IgG3 deficiency was usually expressed also at the cellular level in rectal mucosa, and the proportion of rectal IgG1 cells was significantly correlated ($r = 0{\cdot}90$, $P < 0{\cdot}001$) with the serum IgG1 level (Figs 2a and 3). There tended to be a decreased subclass proportion at the cellular level in the nasal mucosa of patients with serum IgG1 or IgG2 deficiency. Conversely, the median nasal proportion of IgG3 cells was remarkably unaffected by a deficiency of this subclass in serum and rectal mucosa. Interestingly, these seven patients seemed to have raised IgG3 and reduced IgG2 cell proportions in nasal mucosa (Fig. 2a), although this apparent local IgG3 'compensation' was nevertheless strongly correlated with the serum IgG3 level ($r = 0{\cdot}87$, $P < 0{\cdot}002$).

The same discrepant pattern was observed in five patients with combined serum IgG1 and IgG3 deficiency, in two patients with combined serum IgG2 and IgG3 deficiency and in two patients with combined serum IgG1, IgG2 and IgG3 deficiency (Fig. 2b). In all these patients we noted a similar 'compensatory' trend for the IgG3 cell response in nasal mucosa. Further, the almost normal median proportions of nasal mucosa IgG1 cells indicated an accompanying IgG1 cell response in these patients with combined serum IgG subclass deficiencies.

The percentage subclass distributions of mucosal IgG immunocytes determined independently by two investigators (P.B. and D.E.N.) in 24 sections (Fig. 4a) or re-evaluated blindly

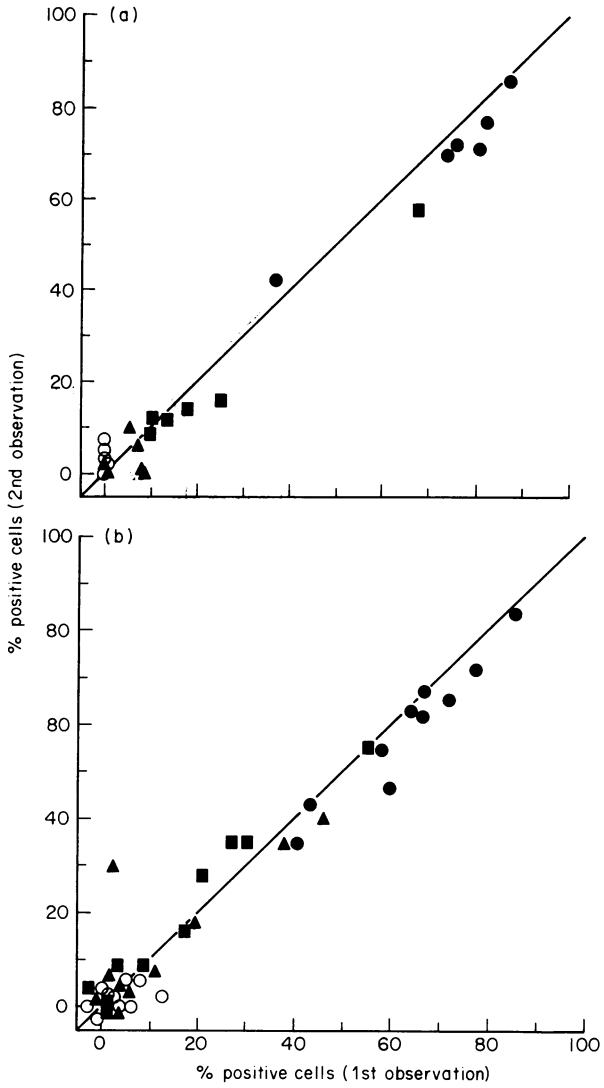

Fig. 4. (a) Test for inter-observer reproducibility. Nasal biopsy specimens from six patients with various IgG subclass deficiencies were examined by immunohistochemistry for the presence of immunocytes producing the four IgG subclasses. Second observer evaluated blindly different sections from the same biopsy specimens 3 years later ($r = 0{\cdot}98$, $P < 0{\cdot}0001$); (b) Test for intra-observer reproducibility. Sections from five nasal and five rectal biopsy specimens were re-evaluated blindly for the four IgG subclasses by the same observer 6 months later ($r = 0{\cdot}97$, $P < 0{\cdot}0001$). ●, IgG1; ▲, IgG2; ■, IgG3; ○, IgG4.

by one of us (D.E.N.) 6 months later in 40 sections (Fig. 4b) were well correlated ($r = 0{\cdot}98$ and $r = 0{\cdot}97$, respectively). It can thus be concluded that the paired immunofluorescence staining method used in this study for *in situ* quantification of immunocytes is highly reproducible.

DISCUSSION

This study showed that the rectal distribution of IgG1 immunocytes was strongly correlated with the serum IgG1 level in patients with single or combined IgG1 subclass deficiencies. There tended to be decreased proportion of the actual subclass at the cellular level in rectal mucosa of patients with serum IgG2 or IgG3 deficiency. All rectal biopsy specimens were obtained

from patients with a histologically normal mucosa. Also, the clinical features of these patients indicated preservation of intestinal immune defence.

Conversely, a remarkable lack of relation was noted between the serum IgG subclass levels and the median proportions of nasal mucosal IgG3 (and sometimes also IgG1) subclass-producing immunocytes. This finding was in agreement with a preliminary study (Brandtzaeg *et al.*, 1986) of a few of the immunodeficient patients included in the present investigation. Thus, a reduced serum IgG3 level was not reflected by a decreased nasal IgG3 cell response, although the mucosal IgG3 'compensation' in the upper respiratory tract was nevertheless strongly correlated with the serum IgG3 level. A similar 'compensatory' trend was noted for both IgG3 and IgG1 in nasal mucosa of patients with a combined serum IgG1 and IgG3 deficiency, and particularly for IgG3 in those with combined IgG2 and IgG3 or IgG1, IgG2 and IgG3 deficiency. It was noteworthy that IgG3-deficient patients, despite a normal serum IgG2-subclass level often showed a strikingly reduced proportion of IgG2 immunocytes in their nasal mucosa. However, this might well be an arithmetic reflection of their increased IgG3 cell proportion.

In contrast to rectal mucosa, histological evaluation of nasal mucosa often showed slight to moderate chronic inflammation in agreement with the predominant clinical finding of rhinopharyngitis. We did not attempt to enumerate the nasal IgG-producing cells in terms of immunocyte density, because of their heterogeneous distribution (Brandtzaeg, 1984). The determination of subclass proportions was performed in areas with abundant glandular elements and in the superficial stroma; the latter compartment often contained numerous lymphoid cells as a reflection of chronic inflammation. It seemed justified to combine our counts obtained from both locations in the mucosa, however, because previously we have been unable to show any difference in the subclass distribution between surface- and gland-associated IgG-producing nasal immunocytes (Brandtzaeg *et al.*, 1987).

The small amounts of IgG that normally reach the gut lumen by passive diffusion are rapidly degraded (Haneberg & Endresen, 1976). IgG is therefore probably of little importance for intestinal surface defence; this may partly explain the preservation of a healthy gut mucosa in our IgG subclass-deficient patients. This conclusion is in agreement with previous reports on patients with more generalized B cell deficiency (hypogammaglobulinaemia), who showed only minor clinical, functional or structural abnormalities in the gut (Eidelman, 1976; Nilssen *et al.*, 1989).

IgG probably plays a more important role at mucosal surfaces in the upper respiratory tract. At least 90% of IgG in nasal fluid has been shown to be of serum origin (Mygind, Weeke & Ullman, 1975). In animal experiments, serum and exocrine IgG molecules have shown identical complement-dependent bactericidal and opsonizing activities (Eddie, Schulkind & Robbins, 1971), and IgG was found to afford immunologic exclusion of soluble antigens in the upper respiratory tract (Stokes, Soothill & Turner, 1975). Mucosal 'leakage' of interstitial IgG is enhanced by inflammatory processes (Brandtzaeg, Fjellanger & Gjeruldsen, 1970; Rossen, Kasel & Couch, 1971), and the classical experiments by Fazekas de St. Groth, Donnelley & Graham (1951) on 'pathotopic potentiation' of local immunity suggested that serum-derived IgG may have an important protective function in the respiratory tract. Indeed, IgG seems to be the major antibody class operating in the lower respiratory tract (Newhouse, Sanchis & Bienenstock, 1976). It is not surprising, therefore, that lack of IgG or one or more of its subclasses has clinical consequences, mainly in the respiratory tract.

The respiratory mucosa is constantly exposed to a heavy bombardment of antigens and mitogens. The nature of the antigen plays an important part in the IgG subclass expression; thus IgG1 and IgG3 antibodies are elicited in T cell-dependent responses by protein antigens like viruses or tetanus toxoid (Papadea & Check, 1989). In contrast, IgG2 antibodies are produced in response to many bacterial antigens including carbohydrates (Shakib & Stanworth, 1980). The IgG subclasses show considerable biological differences with IgG1 and IgG3 being better complement-activating antibodies and more opsonic than IgG2 and IgG4 (Unkeless, Fleit & Mellman, 1981). The discrepancies between the expression of IgG3 subclass deficiency at the two mucosal sites observed in our study may reflect different antigenic and mitogenic loads, for example persistent protein exposure (virus?) of the nasal mucosa which could locally override a B cell maturation defect. Such stimulatory differences would probably also be operating normally and explain the fact that there often is a preference of IgG3 over IgG2 cells in nasal mucosa of subjects with an intact immune system (Brandtzaeg *et al.*, 1986). It remains to be examined whether the apparent 'compensatory' production of IgG3 in the upper respiratory tract of patients with serum deficiency of this subclass might be of any protective significance along with IgG1 and secretory IgA antibodies.

More severe chronic bronchitis, and even chronic lung disease with bronchial asthma or bronchiectasis, occurred in the group with selective IgG1 deficiency, and especially when it was combined with IgG3 deficiency, as described earlier (Oxelius *et al.*, 1986; Bjørkander *et al.*, 1986). The absence of IgG1 is often associated with the same clinical symptoms as in generalized hypogammaglobulinaemia as IgG1 makes up the largest proportion (60–70%) of total IgG (Papadea & Check, 1989). This fact probably explains why these patients often have a history of lung disease and increased susceptibility to pyogenic infections (Schur *et al.*, 1970).

The genetic regulation of IgG subclass expression includes numerous rearrangements and recombinatorial events generating antibody diversity; this complexity allows for error (Papadea & Check, 1989). IgG subclass deficiencies may occur as a result of defects in the constant heavy chain (C_H) genes or in the regulation of their switching on chromosome 14. The gene order on the second ($C_{H\gamma 3}$, $C_{H\gamma 1}$, $C_{H\alpha 1}$) and third ($C_{\gamma 2}$, $C_{\gamma 4}$, $C_{\alpha 2}$) segment (Flanagan & Rabbits, 1982; Conley, Brown & Bartelt, 1987) apparently influences the expression of IgG subclass deficiencies. This may explain the tendency for combined deficiencies to include mainly IgG1 and IgG3 or IgG2 and IgG4 (Hammarström *et al.*, 1984a). B cells switching downstream for one CH gene to another usually deletes the intervening upstream DNA sequences, thus preventing backward switches (Hammarström *et al.*, 1984b). Predominating vectorial switching on the first two gene segments in the sequence $C_{H\mu} \rightarrow C_{H\delta} \rightarrow C_{H\gamma 3} \rightarrow C_{H\gamma 1} \rightarrow C_{H\alpha 1}$ during local immune responses in the upper respiratory tract has been suggested previously by our laboratory; such a mechanism would be in agreement with the normal predominance of IgA1, IgG1 and IgG3 immunocytes in nasal mucosa, in that order

(Brandtzaeg *et al.*, 1986), and a local expansion of IgD along with IgG immunocytes at this site in many patients with IgA deficiency (Brandtzaeg *et al.*, 1979; 1987).

Our main conclusion is that IgG subclass deficiencies, as revealed in serum, were usually expressed also at the cellular level in rectal mucosa. It was remarkable that in patients with low levels of serum IgG3, the proportion of this subclass locally produced in nasal mucosa seemed to be little affected. This apparent local IgG3 compensation was nevertheless positively correlated with the serum IgG3 subclass level. IgG3 and IgG1 antibodies may be of protective significance on the surface of the respiratory mucosa. However, because of their phlogistic properties these subclasses may also be involved in mucosal immunopathology. These immunological aspects are obviously only part of a complex interplay normally taking place in the respiratory tract. Little is known of beneficial or detrimental effects exerted by T cells, macrophages, mast cells, goblet cells and other more poorly defined effector cells present in the mucosa; further studies of patients with various types of immunodeficiency may contribute to a better understanding of these effects.

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