Eur Respir J, 1993, 6, 944–950
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

IgG subclasses in the lungs of patients with interstitial pneumonitis following bone marrow transplantation

H.J. Milburn*, H.G. Prentice**, J.E. Grundy+

IgG subclasses in the lungs of patients with interstitial pneumonitis following bone marrow transplantation. J. Milburn, H.G. Prentice, J.E. Grundy. ©ERS Journals Ltd 1993.

ABSTRACT: Subclasses of immunoglobulin G (IgG) were measured in bronchoalveolar lavage (BAL) fluid and serum from five normal volunteers and 25 bone marrow transplant (BMT) recipients, who developed 32 episodes of pneumonitis. Evidence for local production of the four subclasses was sought, to assess whether any observed deficiency was associated with any particular group of pulmonary infections.

In the normal volunteers, IgG1 and IgG4 could be detected in BAL fluid from all subjects, with evidence for local production of IgG1 in one, and IgG4 in all five. IgG2 could be detected in BAL fluid from one subject, but IgG3 was undetectable in all normal BAL fluid.

The BMT recipients differed from the normal volunteers mainly in the presence of IgG2 and IgG3 in BAL fluid. Furthermore, IgG4 could not be detected in BAL from seven. Furthermore, IgG4 could not be detected in BAL from seven episodes of pneumonitis (six patients). Bacteria, protozoa or fungi alone were isolated from five of these seven lavages, whereas pneumonitis associated with these organisms alone only occurred in 9 of the remaining 25 episodes of pneumonitis (19 patients) where there was also evidence for local production of IgG4. Moreover, 4 out of 7 patients with no detectable IgG4 in lavage developed secondary infections, whilst only 5 out of 19 patients producing IgG4 locally developed secondary infections ($p=0.05$). Although there was individual variation within each group, levels of local production of both IgG1 and IgG4 tended, however, to be higher in patients who died from pneumonitis than in those who recovered, suggesting that this may be a poor prognostic marker.

These results suggest that IgG4 is an important immunoglobulin subclass in the lower respiratory tract, and may have a protective role against bacterial and fungal infections. Excessive local production of IgG1 and IgG4, however, could be associated with a poor prognosis.

Eur Respir J., 1993, 6, 944–950.

* Dept of Respiratory Medicine, Guy's Hospital, London, UK Depts of ** Haematology and + Communicable Diseases, Royal Free Hospital School of Medicine, London, UK.

Correspondence: H.J. Milburn
Chest clinic
Guy's Hospital
St Thomas Street
London SE1 9RT
UK

Keywords: Bone marrow transplant
bronchoalveolar lavage
immunoglobulin subclasses
pneumonitis

Received: March 30 1992
Accepted after revision January 31 1993

We have previously demonstrated that patients with interstitial pneumonitis following bone marrow transplantation (BMT) produce large quantities of all the immunoglobulin classes in their lungs [1]. In the lower respiratory tract, immunoglobulin G (IgG) is the dominant class of antibody, where its opsonizing and complement-binding properties facilitate phagocytosis by macrophages, and promote lytic destruction of microorganisms and other antigens. Human IgG is made up of four subclasses with different biochemical and biological properties, and all of these have been detected in bronchoalveolar lavage (BAL) fluid obtained from normal subjects. Evidence suggests that, although large quantities of IgG1 are present in BAL fluid, it is predominantly IgG4 which is locally produced [2]. This raises the possibility that this subclass, in particular, plays an important role in the lower respiratory tract. However, IgG4 fixes complement poorly, whereas IgG1 and IgG3 (and to a lesser extent IgG2) can activate complement *via* the classical pathway. The predominant subclass response to bacterial polysaccharides, however, is with IgG2 and IgG4, and specific deficiencies of these subclasses in serum have been associated with chronic sino-pulmonary infections [3]. Antibodies produced against protein antigens (*e.g.* viruses) are predominantly IgG1 and IgG3, but chronic stimulation with protein antigens leads to a progressive increase in the proportions of IgG4 [4].

In the present study, we have looked for evidence of local production of the four subclasses of IgG in the lungs of patients with interstitial pneumonitis following BMT, in order to assess whether any observed deficiency is associated with any particular group of pulmonary infections.

Patients

Subclasses of IgG were measured in BAL fluid and serum from 25 recipients of T-cell depleted allogeneic BMT who developed pneumonitis. Nineteen patients were male and six female, and their ages ranged from 12–46 (median 30) yrs. Further patient details are shown in table 1. Ten of these patients had cytomegalovirus (CMV) associated pneumonitis, five had fungal infections (three with *Candida albicans* and two with *Aspergillus fumigatus*), two had bacterial infections (*Staphylococcus aureus* and *Pseudomonas aeruginosa*), two had *Pneumocystis carinii* infections, and one a presumed measles infection. No infectious organism was isolated from the remaining five patients. In addition, seven patients underwent bronchoscopy on a second occasion for recurrence of symptoms. Three of these patients had both first and second episodes of pneumonitis associated with CMV infection, but one had Cryptosporidial oocytes in BAL fluid in addition to CMV, on the second occasion. One patient had *Pneumocystis carinii* initially, but acquired an *Aspergillus fumigatus* infection 12 days later. The remaining three patients had bacterial or fungal infections on both occasions. A total of 32 episodes of pneumonitis were investigated.

Measurements were also made in five normal volunteers who acted as controls.

Table 1. – Patients details

Patient no.		Underlying disease	BAL diagnosis	Time from transplant days
1		AML	CMV	100
2		AML	CMV	234
3		CGL	CMV	133
4		CGL	CMV+*P. carinii*	60
5		AML	CMV	45
6		CGL	CMV+*P. carinii*	115
7		CGL	CMV	73
8		CGL	Idiopathic	357
9		ALL	*C. albicans*	212
10		CGL	Idiopathic	84
11		CGL	*P. carinii*	102
12		ALL	Idiopathic	35
13		AML	Idiopathic	44
14		AML	*A. fumigatus*	101
15		CGL	Measles	737
16		ALL	Idiopathic	150
17		CGL	*A. fumigatus*	128
18		CGL	*C. albicans*	99
19	ep. 1	AML	CMV	98
	ep. 2		CMV	161
20	ep. 1	AML	CMV	58
	ep. 2		CMV+Cryptosporidium	121
21	ep. 1	AML	CMV	84
	ep. 2		CMV	100
22	ep. 1	AML	*C. albicans*	56
	ep. 2		*C. albicans*	86
23	ep. 1	AML	*P. carinii*	87
	ep. 2		*P. carinii* +*A. fumigatus*	99
24	ep. 1	ALL	*S. aureus*	111
	ep. 2		*S. aureus*	184
25	ep. 1	ALL	*P. aeruginosa*	74
	ep. 2		*P. aeruginosa*	84

Pt: patient; ep.: episode; CMV: cytomegalovirus; CGL: chronic granulocytic leukaemia; AML: acute myeloid leukaemia; ALL: acute lymphoblastic leukaemia; BAL: bronchoalveolar lavage.

Methods

Sample collection

Bronchoscopy was performed as described previously [5] using a BFT1 fibreoptic bronchoscope. Bronchoalveolar lavage was carried out using 3×60 ml aliquots of normal saline, buffered to pH 7.4, either in the segment most affected by interstitial shadowing as seen on the chest radiograph, or in the right middle lobe or lingula in cases of either diffuse shadowing or a normal chest radiograph. Sixty to eighty percent of the fluid instilled was recovered and centrifuged at 350×g for 10 min. The supernatant was stored in aliquots at -70°C for future analysis. Serum samples obtained at the time of bronchoscopy were also stored in this way.

Measurement of IgG subclasses:

IgG subclasses and albumin were measured in BAL fluid and serum using radialimmunodiffusion (RID) plates (Serotec). Plates used for albumin were optimized for normal concentrations for serum measurements and low concentrations for BAL measurements. Sera were applied to the standard plates in dilutions according to the manufacturer's instructions, while BAL samples were applied undiluted and the appropriate adjustments made to the results. All plates were incubated at room temperature until the diameter of the undiluted standard reached the appropriate size, indicating complete diffusion. In the case of serum samples this took from 50–100 h, but the lavage samples were incubated for 2–3 weeks before diffusion rings became visible by side lighting. For IgG subclasses in BAL fluid it was necessary to dry the agarose and stain the plates with Coomassie brilliant blue to allow the ring diameter to be measured. Standard curves were constructed using human high and low albumin standards (Serotec), and using human IgG1, IgG2, IgG3 and IgG4 standards (Serotec). These standard curves were made by plotting the square of the diameters of the precipitates formed by the standards against their protein concentrations in $mg \cdot l^{-1}$.

Correction for transudation of serum proteins into BAL fluid

All the immunoglobulin classes are represented in the blood, and so a proportion of these proteins in lung secretions will be derived from the vascular compartment by diffusion across lung tissue, which acts as a semipermeable membrane with respect to proteins in solution. Some

proteins, such as albumin, are derived exclusively from the blood and, therefore, in order to assess whether an immunoglobulin found in lavage fluid is in excess of that which could be present by simple diffusion from serum, an albumin correction was used. The following formula was applied:

$$\frac{[\text{Ig in BAL}]}{[\text{Albumin in BAL}]} \div \frac{[\text{Ig in serum}]}{[\text{Albumin in serum}]}$$

A result >1 implies some local production of antibody in excess of that diffused from serum [6].

BAL plasma cell population

IgG-bearing plasma cells in BAL fluid from the controls and in eight of the transplant lavages were identified using an anti-human IgG conjugated to fluoroscene isothiocyanate (FITC). The preparations were examined using a Zeiss microscope, with epi-illumination and a selective barrier filter for FITC. The immunocytochemical method used has been described in detail previously [7].

Statistical methods

Differences between proportions were assessed by the Chi-squared test, using Fisher's Correction where necessary. Differences between means were assessed by Two Sample t-test for normally distributed data and Mann Whitney U-test for non-parametric data.

Results

Levels of IgG subclasses in serum and BAL fluid

The concentrations of subclasses of IgG in serum and lavage fluid both from controls and from BMT recipients are shown in table 2. Analyses of IgG subclasses in serum specimens from the normal controls disclosed mean concentrations similar to those reported by MERRILL *et al.* [2]. Serum levels were generally slightly lower in the transplant group, but mean lavage levels of IgG1, IgG2 and IgG3 were significantly higher in the BMT recipients compared with normals, although a wide range of levels was found in this group.

Local production of IgG subclasses

Normal volunteers. Measurements of the subclasses of IgG in BAL fluid and serum showed that the major locally produced component of IgG in normal lungs is IgG4 (fig. 1). All normal individuals tested had IgG1 and IgG4 present in the lavage fluid. The BAL/serum ratio for IgG1 approached 1 in four of the five normal controls, but was considerably greater in one individual, indicative of local production. All five normal controls had substantial amounts of locally produced IgG4. IgG2 could only be detected in BAL fluid from one normal volunteer, but the BAL/serum ratio was 1. Again, this could represent some local production of IgG2 in this individual. IgG 3 could not be detected by RID in BAL fluid of any normal volunteer, but normal levels were detectable in serum.

Patients with pneumonitis. The BMT recipients with pneumonitis were quite different from normal volunteers in the pattern of IgG subclasses present in the lavage fluid. Although IgG1 could be detected in BAL fluid from all patients (table 3), local production of antibody could only be demonstrated in 9 of 13 episodes of pneumonitis associated with CMV and in 17 of 19 in which CMV was not detected. Many patients were, however, also producing IgG2, IgG3 and IgG4 in their lungs (table 3).

IgG4 could not be detected in lavage fluid from one patient with CMV, one with CMV and *Pneumocystis carinii,* and in five other patients with *Pneumocystis carinii* and fungal infections. Of the seven patients with no detectable IgG4 in BAL fluid, five had bacterial, protozoal or fungal infections, whereas, only 9 out of 19 of patients

Table 2. – Concentrations of immunoglobulin G (IgG) subclasses in lavage fluid and serum

	IgG1	IgG2	IgG3	IgG4
Serum				
Controls	102±12.5×10²	40±3.2×10²	75.5±2×10	29.3±3.5×10
BMT	90.1±7.1×10²	30.1±2×10²	67.7±7.1×10	23±3×10
	NS	p=0.05	NS	NS
Lavage				
Controls	31.1±8.9	1.64 (one patient)	ND in any specimen	23±1.2
BMT	153.6±21	3.2±0.3	25.4±3.5	24.8±2.7
	p=0.025	p<0.025	p<0.01	NS

Concentrations of subclasses are expressed in $mg \cdot l^{-1}$. Data represent mean±SEM. BMT: bone marrow transplant recipients; ND: not detected; NS: no significant difference by Mann Whitney U-test.

producing IgG4 in the lung had bacterial, protozoal or fungal infections, suggesting that there may be some association between IgG4 in the lung and defence against bacterial and fungal infections. Only five of the 19 patients producing IgG4 locally went on to develop a secondary infection (two with CMV, one with *Candida albicans* and two with bacterial infections), whereas four of the seven patients with no detectable IgG4 in lavage developed secondary infections (one with CMV, one with *Candida albicans* and two with *Aspergillus fumigatus*). This approached statistical significance with p=0.05 (Fisher's Exact Test). IgG4 levels were generally high in patients with virus infections (all CMV except for one presumed measles infection) and patients in whom no infectious organism was isolated. In addition, there were lower levels of local production of IgG1 in the patients with CMV-associated pneumonitis, compared with the others. There were no other differences in IgG subclasses in BAL fluid between the CMV positive and CMV negative groups.

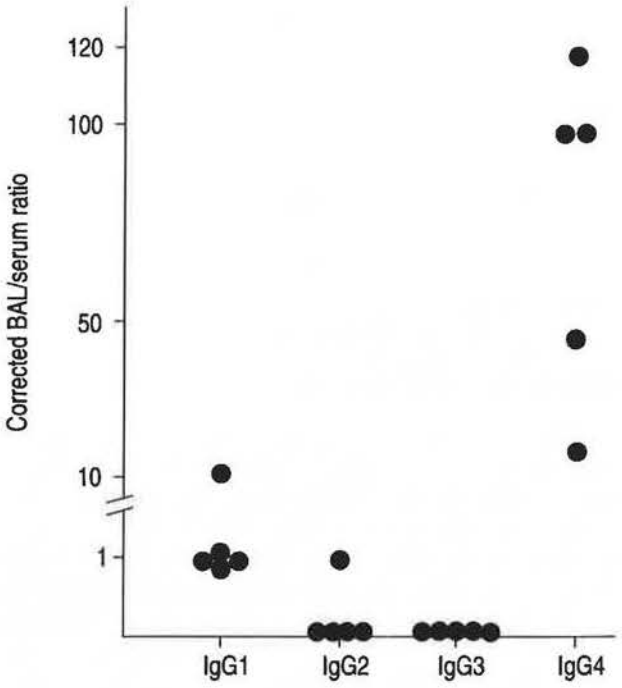

Fig. 1. – Corrected BAL/serum ratios of IgG subclasses in normal volunteers. BAL: bronchoalveolar lavage; IgG: immunoglobulin G.

IgG subclasses were measured in lavage and serum in seven of the BMT recipients who underwent bronchoscopy on two separate occasions. The results of each subclass of IgG were very different on each occasion for each patient (table 4). The levels of IgG4 were extremely high in lavage relative to serum in those patients who died from a second episode of pneumonitis.

BAL plasma cell population

In the eight BMT lavages studied, 2–5% of the total cell population were IgG-bearing plasma cells. In some specimens, many more cells fluoresced, but these were macrophages containing IgG. There was no fluorescence, even in the macrophages, in the normal control lavages.

Immunoglobulin subclass production and survival

Ten of the patients investigated for immunoglobulin production in the lung survived 12 episodes of pneumonitis, and two more survived a first episode but died after a second. The cut-off time chosen for survival was 30 days, as all patients who died from their pneumonitis did so within 30 days of bronchoscopy although the majority who died did so during the first two weeks (12 out of 15 patients). Fifteen patients died after 18 episodes of pneumonitis.

There was a tendency for higher levels of local production of IgG1 and IgG4 in patients who died from

Table 3. – Detection and local production of immunoglobulin G (IgG) subclasses in the lung

Number of pneumonitis episodes	IgG1		IgG2		IgG3		IgG4	
	Det	Prod	Det	Prod	Det	Prod	Det	Prod
BMT: virus +ve (n=14)	14	10	13*	6	9⁺	9⁺	12	12
BMT: virus -ve (n=18)	18	15	15*	6	13*	12⁺	13	13
BMT: survived (n=14)	14	12	13	5	9	9	11	11
BMT: died (n=18)	18	14	15	7	13	12	14	14
Normal volunteers (n=5)	5	1	1	0	0	0	5	5

Data shown are number of pneumonitis episodes with detectable (Det) IgG subclasses in bronchoalveolar lavage fluid, and number of patients with evidence for local production (Prod) of IgG subclasses, *i.e.* corrected BAL: serum ratios >1. Virus +Ve: episodes of pneumonitis associated with virus in BAL fluid; 13 with CMV and 1 with measles. Significance of comparisons made with results in normal volunteers is shown by ⁺: p=0.05; *: p<0.01 (by Fisher's Exact Test). BMT: bone marrow transplant recipients.

Table 4. – Local production of immunoglobulin G (IgG) subclasses in the lung

Pt no.	Diagnosis	IgG1	IgG2	IgG3	IgG4	Outcome
19	1. CMV	57	4.3	135	500	Recovered
	2. CMV	1.2	0.1	UD	20.8	Recovered
20	1. CMV	180	14.4	825	110	Recovered
	2. CMV + Crypto	2.4	0.2	7	20	Died
21	1. CMV	2.9	28.6	UD	UD	*
	2. CMV	297	13.4	273	1000	Died
22	1. *C. albicans*	4.6	0.5	67	23	Recovered
	2. *C. albicans*	1.0	1.0	UD	UD	Recovered
23	1. *P. carinii*	2.5	UD	21	UD	*
	2. *P. carinii* + *A. fumigatus*	148	8.6	5	1667	Died
24	1. *S. aureus*	1.0	0.2	4.9	22	Recovered
	2. *S. aureus*	646	16.7	459	1250	Died
25	1. *P. aeruginosa*	9.1	UD	UD	1250	*
	2. *P. aeruginosa*	3.2	0.5	UD	34	Died

BMT recipients with pneumonitis who underwent bronchoscopy on two separate occasions. Figures shown are BAL/serum ratios corrected for albumin. Pt: patients; CMV: cytomegalovirus; Crypto: cryptosporidium; BAL: bronchoalveolar lavage; UD: undetected; *: patient bronchoscoped twice during same episode.

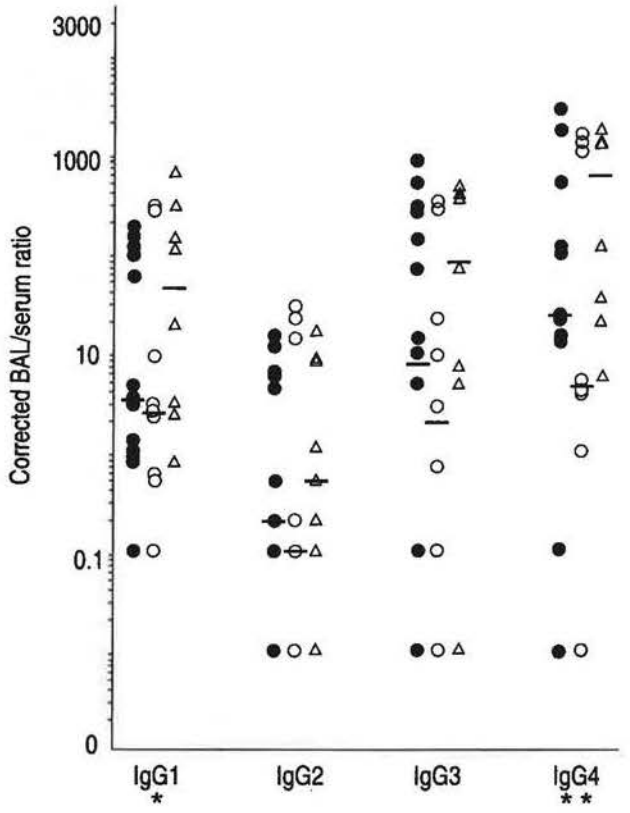

Fig. 2. – Corrected BAL/serum ratios for IgG subclasses in patients with pneumonitis. Comparison between patients who survived an episode of pneumonitis (●); patients who survived for between 10–30 days following diagnosis (○); and patients who died within 10 days of diagnosis of pneumonitis (Δ); — : corresponds to geometric mean. Ratios between groups were significantly different for IgG1 and IgG4. *: $p<0.005$; **: $p<0.01$. For abbreviations see legend to figure 1.

pneumonitis, compared with those who survived (fig. 2). This difference was apparent in patients who died within 10 days of bronchoscopy, whereas those who survived for between 10–30 days had BAL/serum ratios for IgG1 and IgG4 similar to the survivors. Local production of IgG3 was also higher in the group who died within 10 days, but this did not reach statistical significance. There was no difference in local production of IgG2. There was a substantial individual variation within each group, from no evidence for local production to BAL/ serum ratios of >1,000. Taking the group who died as a whole, extremely high BAL/serum ratios (>100 for IgG1) were found in 5 out of 15 patients who died within 30 days of diagnosis and 3 out of 12 patients who survived for more than 30 days from 14 episodes of pneumonitis. Ratios >1,000 for IgG4 were found in 6 out of 15 patients who died and 2 out of 14 of the survivors.

Discussion

Following allogeneic BMT for haematological malignancies, patients who develop pneumonitis are able to produce IgG in their lungs, or alternatively have a method for concentrating the protein [1]. The data reported in the present study demonstrate that both the proportions and quantities of subclasses of IgG are abnormal in the lungs of these patients and may be related to the outcome of pneumonitis.

Immunoglobulins detected in pulmonary secretions may either have been derived from the blood, or may be locally produced by cells within lung tissue. Some proteins, however, are derived exclusively from the blood, and for these, the ratio of secretion:serum concentration is inversely proportional to protein size [8]. Such proteins

include albumin and ceruloplasmin, and the concentration of these in secretions will depend on serum concentration and the degree of inflammation in the lung producing protein transudation [8, 9]. To obtain an independent measure of the permeation of proteins from blood into BAL fluid, we measured albumin in serum and lavage, and were thus able to calculate the proportion of individual IgG subclasses present in BAL fluid above levels expected by diffusion alone. RENNARD *et al.* [10] proposed the use of urea instead of albumin for estimating proportions of proteins present in BAL fluid, but there have been conflicting reports on its reliability as an independent marker [11–14]. There is still no clear consensus on the best internal marker of dilution of BAL fluid, and albumin remains the marker most commonly used.

Even with serum levels there remains uncertainty on the true normal values of the immunoglobulin subclasses and how best to measure them [15]. Normal values vary widely within the population, and may change within a given individual from time to time, and as a result of relatively minor stimuli [16].

The pattern of IgG subclasses found in the lavage fluid from the normal volunteers in this study was comparable with the data of MERRILL *et al.* [2], with IgG4 being the main locally produced subclass in the lower respiratory tract. We were, however, unable to detect IgG3 in BAL fluid from the normal volunteers, whereas Merrill *et al.* found raised levels in smokers relative to serum. One possibility for this discrepency may reflect the fact that only one of our controls and one patient smoked, or alternatively could be related to the relative sensitivities of the different assays used. In a more recent study by OUT *et al.* [17], however, the levels of IgG3 found in BAL fluid from normals was relatively much lower than in serum, more in accordance with our data than with those of MERRILL *et al.* [2].

The BMT recipients differed from the normal volunteers mainly in the presence of IgG2 and IgG3. Furthermore, IgG4, the subclass commonly found in normal BAL fluid, could not be detected in some lavages from the BMT recipients. These differences were magnified when albumin measurements were used to correct for simple diffusion and to determine the degree of local production or active concentration of this subclass in lung secretions. A proportionately greater number of patients with no detectable IgG4 in BAL fluid had bacterial, protozoal or fungal infections, compared with those patients with local production of IgG4. Furthermore, those patients deficient in IgG4 in the lavage also had a greater tendency to develop secondary infections, often with fungi. Very low levels of serum IgG2 and IgG4 have been found in patients with chronic mucocutaneous candidiasis who also develop severe lung infections, particularly with encapsulated organisms [18]. In addition, LOH *et al.* [19] found that high proportions of children with Down's Syndrome, who were also susceptible to infections had low serum levels of IgG4. IgG4 deficiency has also been demonstrated in patients susceptible to recurrent sinopulmonary infections [3]. It is possible, therefore, that IgG4 may have an important protective role in the lower respiratory tract, particularly against bacterial and fungal infections. Alternatively, an IgG4 deficiency may be one manifestation of a B-cell abnormality, which also gives rise to other defects of antibody-mediated immune responses. This possibility is supported by the observation that IgG4 deficiency is found in a number of conditions not necessarily associated with increased susceptibility to infection, for example systemic lupus erythematosus, ataxia telangiectasia, epilepsy and autoimmune cyto-poenias [20, 21], but in which an immunoregulatory abnormality is present. IgG4 does not activate complement and binds poorly to Fc receptors of phagocytic cells. It would, therefore, be surprising if it were to have anything but a minor direct role to play in antibody responses to bacteria, but could be involved in blocking bacterial adherence [22].

Infection or vaccination with various viruses or vaccines has been shown to induce the preferential production of at least one of the four subclasses of IgG, with specific patterns seen for herpes simplex virus [23], varicella zoster virus [23], and respiratory syncitial virus [24]. Primary CMV infection results in a predominantly IgG1 and IgG3 response [25], but an additional IgG4 response has been found in approximately 30% of BMT recipients with primary CMV infections [26]. In the BMT recipients with pneumonitis, there was no difference in the overall levels of subclasses produced in the lung between those patients with virus infections (all CMV except for one patient with a presumed measles infection) and those patients with other infections or idiopathic pneumonitis, except for the reduced or absent IgG4 levels in those with bacterial or fungal infections. It is possible, however, that measurement of virus specific levels of IgG subclasses might demonstrate a difference between the groups.

It might be expected that the ability to mount a humoral immune response in the lung following bone marrow transplantation would be an important factor in recovery from opportunistic infections. However, we have previously found no evidence that local production of either total or virus specific immunoglobulins [1, 27] contributed towards survival. Moreover, in the present study, there was a tendency for increased local production of the subclasses IgG1 and IgG4 in patients who died from pneumonitis. This may mean that patients who died were unable to utilize the locally produced antibody appropriately, or that a massive outpouring of antibody was a final attempt at recovery. A further possibility is that antibody-mediated damage to cells may have contributed to the pathology of pneumonitis. It has been suggested that IgG4 antibodies might be important effector molecules in asthmatic and hypersensitivity conditions [28]. Levels of IgG4 have been found to be increased in lung lavage fluid from patients with pigeon breeders' hypersensitivity pneumonitis [29]. In immediate-type allergic reactions, it is possible that subtypes of IgG4 antibodies sensitize mast cells and basophils [30], but OUT *et al.* [17] found no relationship between the local production of IgG subclasses and clinical parameters, in a group of asthmatics, nor could they find any specific role for one subclass. Other workers argue for a blocking role for IgG4 [31], and IgG4 has been found to moderate the size and complement fixing properties of mixed immune complexes

[32]. The precise function of IgG4 remains unclear, but may include prevention of bacterial adherence and/or blocking of the inflammatory reaction in the lower respiratory tract.

In conclusion, this study demonstrates an imbalance of local production of the subclasses of IgG in the lungs of BMT recipients with pneumonitis. In particular, different patterns were found from normals, and IgG4 could not be detected in the lower respiratory tract in patients with fungal or bacterial infections. Furthermore, excessive local production of both IgG1 and IgG4 tended to be associated with a poor prognosis. These results may represent a response to infection, or could reflect an immunoregulatory abnormality, either following marrow transplantation or induced by the pulmonary infection itself.

References

1. Milburn HJ, Grundy JE, du Bois RM, Prentice HG, Griffiths PD. – Humoral immune responses in the lung of bone marrow transplant recipients studied by bronchoalveolar lavage. *Clin Exp Immunol* 1988; 72: 309–314.
2. Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. – Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects. *Am Rev Respir Dis* 1985; 131: 548–587.
3. Beck CS, Heiner DC. – Selective immunoglobulin G_4 deficiency in recurrent infections of the respiratory tract. *Am Rev Respir Dis* 1981; 124: 94–96.
4. Skvaril F. – IgG subclasses and viral infections. *Monogr Allergy* 1986; 19: 134–143.
5. Milburn HJ, Prentice HG, du Bois RM. – The role of bronchoalveolar lavage in the evaluation of interstitial pneumonitis in bone marrow transplant recipients. *Thorax* 1987; 42(10): 766–772.
6. Wiggins J, Stockley RA. – Variability in sputum sol phase proteins in chronic obstructive bronchitis: the value of using albumin for standardisation. *Am Rev Respir Dis* 1983; 128: 60–63.
7. Milburn HJ. – Interstitial pneumonitis in bone marrow transplant patients studied by bronchoalveolar lavage. MD Thesis, University of London, 1991; pp. 160.
8. Stockley RA, Mistry M, Bradwell AR, Burnett D. – A study of plasma proteins in the sol phase of sputum from patients with chronic bronchitis. *Thorax* 1979; 34: 777–782.
9. Out TA, Jansen HM, Van Steenwijk RP, *et al.* – ELISA of ceruloplasmin and $alpha_2$-macroglobulin in paired bronchoalveolar lavage fluid and serum samples. *Clin Chim Acta* 1987; 165: 277–288.
10. Rennard SI, Basset G, Lecossier D, *et al.* – Estimation of volume of epithelial lining fluid recovered by lavage using urea as a marker of dilution. *J Appl Physiol* 1986; 60(2): 532–538.
11. Marcy TW, Merrill WW, Rankin JA, Reynolds HY. – Limitation of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. *Am Rev Respir Dis* 1987; 135(6): 1276–1280.
12. Seitsema K, Effros RM, Siu ST, Mason GR. – Solute concentrations of bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1986; 133: A20.
13. Weinberg SE, Kelman JA, Elson NA. – Bronchoalveolar lavage in interstitial lung disease. *Ann Intern Med* 1978; 89: 459–466.
14. Jones KP, Edwards JH, Reynolds SP, Peters TJ, Davies BH. – A comparison of albumin and urea as reference markers in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Eur Respir J* 1990; 3: 152–156.
15. Levinsky RJ. – IgG Subclass Deficiencies. International Congress and Symposium Series. London, Royal Society of Medicine, 1989.
16. Jefferis R, Kumararatne DS. – Selective IgG subclass deficiency: quantification and clinical relevence. *J Clin Exp Immunol* 1990; 81: 357–367.
17. Out TA, Van der Graaf EA, Van den Berg NJ, Jansen HM. – IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. *Scand J Immunol* 1991; 33: 719–727.
18. Bentur L, Nisbet-Brown E, Levison H, Roifman CM. – Lung disease associated with IgG subclass deficiency in chronic mucocutaneous candidiasis. *J Paediatr* 1991; 118(1): 82–86.
19. Loh RKS, Harth SC, Thong YH, Ferrante A. – IgG subclass deficiency and predisposition to infection in Down's Syndrome. *Paediatr Infect Dis J* 1990; 9(8): 547–551.
20. Oxelius VA. – Immunoglobulin G (IgG) subclasses and human disease. *Am J Med* 1984; 76: 7–18.
21. Heiner DC, Lee SI, Short JA. – IgG4 subclass deficiency syndromes. *Monogr Allergy* 1986; 20: 149–156.
22. Margni RA, Binaghi RA. – Non-precipitating asymmetric antibodies. *Ann Rev Immunol* 1988; 6: 535–554.
23. Sundqvist VA, Linde A, Wahren B. – Virus-specific immunoglobulin G subclasses in herpes simplex and varicella zoster virus infections. *J Clin Microbiol* 1984; 20: 94–98.
24. Watt PJ, Zardis M, Lambden PR. – Age-related IgG subclass response to respiratory syncitial virus fusion protein in infected infants. *Clin Exp Immunol* 1986; 64(3): 503–509.
25. Linde GA, Hammarstrom L, Persson MA, *et al.* – Virus-specific antibody activity of different subclasses of immunoglobulins G and A in cytomegalovirus infections. *Infect Immun* 1983; 42: 237–244.
26. Wahren B, Linde A, Sundqvist VA, *et al.* – IgG subclass-specific CMV reactivity in bone marrow transplant recipients. *Transplantation* 1984; 38: 479–483.
27. Milburn HJ, Grundy JE, du Bois RM, Prentice HG, Griffiths PD. – Is the measurement of virus-specific antibody in the lungs of transplant recipients of diagnostic or prognostic value? *J Med Virol* 1988; 26: 570–575.
28. Gwynn CM, Ingram J, Almonsarwi T, Stanworth DR. – Bronchial provocation tests in atopic patients with allergen-specific IgG4 antibodies. *Lancet* 1982; i: 254–256.
29. Calvanico NJ, Ambegaonkar SP, Schlueter DP, Fink JN. – Immunoglobulin levels in bronchoalveolar lavage fluid from pigeon breeders. *J Lab Clin Med* 1980; 96: 129–140.
30. van der Zee JS, Aalberse RC. – The role of IgG. *In*: Lessof MM, Lee TH, Kemeny DM, eds. Allergy. 2nd edn. Chichester, Wiley Medical Publications, 1987; pp. 49–69.
31. Aalberse RC, Dieges PH, Knul-Bretlova V, *et al.* – IgG4 as a blocking antibody. *Clin Rev Allergy* 1983; 1: 289–302.
32. van der Zee JS, van Swieten P, Aalberse RC. – Serological aspects of IgG4 antibodies. II. IgG4 antibodies form small non-precipitating immune complexes due to functional monovalency. *J Immunol* 1986; 137: 3556–3571.