

High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens

R. DJURUP and H.-J. MALLING

*The Laboratory of Medical Allergology and the Allergy Clinic,
Medical Department TTA, Rigshospitalet, Copenhagen*

*(Received in original form 15 December 1986 and in second revised form 8 April 1987;
accepted for publication 8 April 1987)*

Summary

We have analysed all available data on the relationship between IgG4 Ab level and clinical effect of immunotherapy (IT) with inhalant allergens. The data from three of the seven independent studies could, without reservations, be analysed by a joint statistical analysis. We found that late high IgG4 Ab level, measured at the end of IT, was strongly associated with treatment failure ($P = 6.54 \times 10^{-5}$; $n = 67$). The ratio of risks for treatment success in the group with late low IgG4 Ab level was 1.82, whereas the ratio of risks for treatment failure in the group with late high IgG4 Ab level was 11.4. The data from a fourth, presumably comparable, study further supported the existence of an association between high IgG4 Ab level and treatment failure. In contrast, two other studies found that high mean IgG4 Ab level was associated with good clinical response. Possible reasons for this apparent discrepancy are discussed. We also found that early high IgG4 Ab level, measured within 3 months after initiation of IT, was strongly associated with treatment failure after 1–2 years of IT ($P = 1.05 \times 10^{-4}$; $n = 30$). The sensitivity and specificity of early high IgG4 Ab level as indicator for treatment failure was 100% and 83%, respectively. At the prevalences found in the present study, the predictive value of early high IgG4 Ab level for treatment failure was 0.6, whereas the predictive value of early low IgG4 Ab level for treatment success was 1.00.

Introduction

Based mainly on the findings by Devey *et al.* [1] and by van der Giessen *et al.* [2] that IgG antibodies formed during immunotherapy (IT) with inhalant allergens are predominantly of the IgG4 subclass, it has been suggested that IgG4 antibodies are the most important 'blocking antibodies' [3]. However, the evidence for this assumption is

Correspondence: René Djurup, MD, Ph.D., Medical Department, TTA Rigshospitalet, Tagensvej 20, DK-2200 Copenhagen N, Denmark.

at best circumstantial, as recently pointed out by Aalberse *et al.* [3] who found an increased frequency of treatment failures in patients with very high level of IgG4 Ab. A similar observation was made by Etievant *et al.* [4]. Presumably, Etievant *et al.* were the first to suggest that IgG4 Abs formed during IT could be sensitizing in some patients. Before that time, Parish [5] had shown the existence of human IgG anaphylactic antibodies, and experiments by Stanworth and Smith [6] had indicated that these were likely to be of the IgG4 subclass.

In a study from our own clinic [7] we found a positive correlation, significant at the 0.10 level, between IgG4 Ab level and symptom score after both 1 and 2 years of IT with grass pollen. In a recent study (Malling and Djurup, in prep.) we again found a weak association between high IgG4 Ab level after 1 year of IT with *Cladosporium herbarum* and treatment failure. We, therefore, thought it of interest to perform a joint statistical analysis of all data available on the relationship between IgG4 Ab level and clinical outcome of IT with inhalant allergens.

Subjects and methods

Studies of the relationship between IgG4 Ab level and clinical outcome

To our knowledge only seven studies [1,3,4,7-9, Malling and Djurup (in prep.)] have attempted to correlate or associate the level of IgG4 Abs with the clinical outcome of IT with inhalant allergens. These studies are not entirely comparable and only the data presented by Aalberse *et al.* [3], Djurup and Østerballe [7] and Malling and Djurup (in prep.) are in a form that indisputably permits their inclusion into a joint statistical analysis. As will be discussed, the data from Etievant *et al.* [4] could, with some reservations, be included too. In some studies [1,8,9] neither data on IgG4 Ab level and clinical result nor exact probabilities for the statistical relationships were given. It is, therefore, not possible to include these studies in a joint statistical analysis [10]. However, as Nakagawa [8] and Hedlin *et al.* [9] present data in favour of a beneficial role of high IgG4 Ab level, whereas the result of the joint analysis strongly suggests an increased frequency of treatment failures in patients with high IgG4 Ab levels, we shall discuss their findings extensively. Below we briefly describe the studies included in the joint analysis.

Study I [3]. In this study thirty-seven hay fever patients were immunized for more than 2 years with grass pollen extract. No data on allergen form (e.g. aqueous or alum-adsorbed) or injection schedule (e.g. single injections or clustered injections) were given. IgG4 Abs to grass pollen were measured by an IgG4 RAST calibrated by a human reference serum. Results were stated as per cent of the binding found with the reference serum. No data on precision were given. The authors stated that four of five treatment failures (as judged by clinical evaluation) had high IgG4 Ab levels (and high IgG1 Ab levels too). Based on this statement and the results presented in Fig. 8 [3] we define 'high IgG4 Ab level' as binding greater than 100% of the reference serum. This Figure [3] shows that seven patients had high IgG4 Ab levels after more than 2 years of IT and thirty patients had low levels. Four of the seven patients with high levels failed to improve, whereas only one patient with a low level failed. No data on early IgG4 Ab levels or the final clinical results were presented.

Study II [7]. In this study nineteen patients were immunized with single injections of an alum-adsorbed extract of grass pollen for more than 2 years. IgG4 Ab levels were measured by an IgG subclass RAST, calibrated by a human reference serum in

Table 1. Association between late IgG4 Ab level and treatment result in study I

	Treatment success	Treatment failure	
High IgG4 Ab level	3	4	7
Low IgG4 Ab level	29	1	30
	32	5	37

$$G_{\text{adj}} = 9.21; P = 2.41 \times 10^{-3}.$$

arbitrary units (U/ml). Interassay coefficient of variation (CV) was between 15% and 22%. In the present study we arbitrarily define high IgG4 Ab level as more than 17 U/ml at the end of IT and as more than 8 U/ml after 12 weeks of IT [7]. Patients having more than seventy-six points in the symptom score at the end of IT were classified as having a 'poor clinical result' [7].

Study III [(Malling and Djurup, in prep.)]. A detailed account of this study will be presented elsewhere. Here, we shall only present data on the statistical relationships between IgG4 Ab levels and clinical outcome.

Eleven asthmatic patients were immunized for 1 year with an aqueous extract of *C. herbarum*. The dose-increase phase was performed as clustered injections at fortnightly intervals and the maintenance dose was a single monthly injection [11]. IgG4 Abs were quantitated by a modification of the IgG subclass RAST described by Djurup *et al.* [12]. As most patients had significant amounts of specific Abs in one or more subclasses we found it logical to use the change in subclass level, rather than the absolute level, as the parameter for the response to the IT. Accordingly, we define 'high IgG4 Ab level' as an increase on more than 10 U/ml after 1 year of IT and as an increase on more than 5 U/ml after 6 weeks of IT (Malling and Djurup, in prep.). The clinical condition after 1 year of IT was classified as 'improved', 'unchanged', or 'deteriorated' [11]. As the *Cladosporium* spore count was two to three times higher for 7 out of 10 weeks in the 10-week registration period (peak *Cladosporium* season) following IT than in the preceding pre-treatment season, we considered patients with improved or unchanged clinical condition to be successfully treated [11].

Statistics

Associations between IgG4 Ab levels and clinical outcome were evaluated by 2×2 contingency tables. As the marginal totals were not fixed we used the *G*-test of independence with Williams' correction to evaluate the association [13]. The adjusted *G* (G_{adj}) is approximately distributed as chi-square (χ^2) with one degree of freedom [13].

Table 2. Association between late IgG4 Ab level and treatment result in study II

	Treatment success	Treatment failure	
High IgG4 Ab level	4	3	7
Low IgG4 Ab level	11	1	12
	15	4	19

$$G_{\text{adj}} = 2.71; P = 9.97 \times 10^{-2}.$$

Table 3. Association between late IgG4 Ab level and treatment result in study III.

	Treatment success	Treatment failure	
High IgG4 Ab level	3	2	5
Low IgG4 Ab level	6	0	6
	9	2	11

$$G_{\text{adj}} = 2.93; P = 8.69 \times 10^{-2}.$$

Correlation between early and late IgG4 Ab levels was assessed by Spearman's rank correlation coefficient (r_s). Significance of r_s was evaluated by Kendall's method [14].

Ratios of risks were calculated as described by Armitage [15]. Predictive values were calculated as described by Sheps and Schechter [16].

Exact probabilities corresponding to observed G_{adj} (χ^2) and t values were calculated by use of Hewlett Packard's 'General Statistics Program' to the computer HP-86.

Associations and correlations with a two-tailed stochastic probability equal to or less than 0.05 were considered statistically significant.

Results

Associations between late IgG4 Ab level and final clinical outcome

In study I late high IgG4 Ab level was found to be highly significantly associated with treatment failure ($P = 2.41 \times 10^{-3}$) (Table 1). In study II the association was not significant ($P = 9.98 \times 10^{-2}$) (Table 2). Neither was the association found in study III significant ($P = 8.69 \times 10^{-2}$) (Table 3). In Table 4 we have pooled the data from studies I–III. By this joint analysis the association between late high IgG4 Ab level and treatment failure was found to be highly significant ($P = 6.54 \times 10^{-5}$). The ratio of risks (RR) for success in the group with late low IgG4 Ab level (calculated on basis of the data in Table 4) was 1.82, whereas the RR for failure in the group with late high IgG4 Ab level was 11.4.

Associations between early IgG4 Ab level and final clinical outcome

In study II (Table 5) we found a highly significant association between early high IgG4 Ab level (measured after 12 weeks of IT) and treatment failure ($P = 1.34 \times 10^{-3}$). In study III (Table 6) the association between early high IgG4 Ab level (measured as the increase during the first 6 weeks of IT) and treatment failure after 1 year almost reached

Table 4. Association between late IgG4 Ab level and treatment result found by pooling data from studies I, II, and III

	Treatment success	Treatment failure	
High IgG4 Ab level	10	9	19
Low IgG4 Ab level	46	2	48
	56	11	67

$$G_{\text{adj}} = 15.94; PO = 6.54 \times 10^{-5}.$$

Table 5. Association between early IgG4 Ab level and final clinical outcome in study II

	Treatment success	Treatment failure	
High IgG4 Ab level	2	4	6
Low IgG4 Ab level	13	0	13
	15	4	19

$$G_{\text{adj}} = 10.28; P = 1.34 \times 10^{-3}.$$

significance ($P = 5.16 \times 10^{-2}$). When the data from the two studies were combined, the significance of the association manifested itself very clearly ($P = 1.05 \times 10^{-4}$) (Table 7).

The RR for treatment success in the group with early low IgG4 (calculated from the data in Table 7) is 2.5. The RR treatment failure in the group with early high IgG4 Ab level cannot be calculated as the denominator would be zero.

Using Table 7 as a decision matrix, the sensitivity and specificity of early high IgG4 Ab level as an indicator for treatment failure is 100% and 83%, respectively [16].

Assuming that the prevalences of patients with early high IgG4 Ab levels (0.33) and with early low IgG4 Ab levels (0.67), which appears from Table 7, are representative, the predictive value of early high IgG4 Ab level for treatment failure would be 0.6, whereas the predictive value of early low IgG4 Ab level for treatment success would be 1.00.

Correlation between early and late levels of IgG4 Abs

The correlation between early and late IgG4 Ab levels found in studies II + III was $r_s = 0.595$ ($n = 30$; $P = 2.60 \times 10^{-4}$).

Discussion

Association between late high IgG4 Ab level and treatment failure

The joint analysis of data on the relationship between late IgG4 Ab level and treatment failure from three independent studies of different regimens of IT with two different inhalant allergens provides powerful evidence that late high IgG4 Ab level is strongly associated with treatment failure. The results presented in a fourth study [4] further support the existence of such general association. Etievant *et al.* [4] studied the effect of IT with house dust in twenty-three patients with rhinitis (four patients) or asthma (nineteen patients). Data on IgG4 Ab level and clinical effect were given for fifteen patients. As the authors neither stated the duration of IT nor the time for measurement

Table 6. Association between early IgG4 Ab level and final clinical outcome in study III

	Treatment success	Treatment failure	
High IgG4 Ab level	2	2	4
Low IgG4 Ab level	7	0	7
	9	2	11

$$G_{\text{adj}} = 3.79; P = 5.16 \times 10^{-2}.$$

Table 7. Association between early IgG4 Ab level and final clinical outcome found by pooling data from studies II and III

	Treatment success	Treatment failure	
High IgG4 Ab level	4	6	10
Low IgG4 Ab level	20	0	20
	24	6	30

$$G_{\text{adj}} = 15.04; P = 1.05 \times 10^{-4}.$$

of IgG4 Ab we preferred not to include them in the statistical analysis proper. However, if one makes the reasonable assumption that IgG4 Ab levels (which were measured only once) were measured late in the course of IT and at the time when the clinical evaluation was performed, it becomes possible to compare them with the results from studies I, II, and III. Defining IgG4 Ab levels greater than 2.00 as high, four of five treatment failures and three of ten treatment successes had late high IgG4 Ab level (Table 1 in [4]) ($G_{\text{adj}} = 3.13$; $P = 7.69 \times 10^{-2}$). Even if our assumption concerning the time for measurement of IgG4 Ab level is wrong, the results of this study clearly support our hypothesis that treatment failures preferentially befall patients with high IgG4 Ab levels.

The result of the above joint analysis apparently stands in contrast to the finding by Nakagawa [8] and by Hedlin *et al.* [9].

Nakagawa studied seventy patients with rhinitis undergoing IT with house dust mite. Treatment was found to be effective in twenty-eight patients, slightly effective in twenty-six patients, and ineffective in thirteen patients. Results of the clinical evaluation of the remaining three patients were not given. Nakagawa found that the increase in mean level of IgG4 Ab was significantly higher ($P < 0.05$) in the group with good effect ($\bar{x} \pm \text{s.d.} = 3.98 \pm 3.15$) than in the group with no effect ($\bar{x} \pm \text{s.d.} = 1.95 \pm 1.55$). However, (from Fig. 3 in [8]) it appears that twelve, twenty-four and forty-five patients left the study within 6, 12, and 24 months, respectively. As sixty-seven patients were evaluated clinically, the effectiveness and IgG4 Ab level must have been assessed at different times for different patients. Thus, the study is not comparable to the other studies mentioned above and underlying the statistical analysis presented in this paper. Furthermore, assuming—in agreement with most available knowledge—that both clinical effectiveness and IgG4 Ab level increases with increasing duration of IT, the relationship between clinical result and IgG4 Ab level may have been biased in Nakagawa's study [8]: thus, patients who stop IT early are likely to display both low IgG4 Ab level and poor clinical outcome.

The study by Hedlin *et al.* [9] included twenty-three patients treated with either cat- or dog-dander extract. It was found that the group (thirteen patients) who improved had significantly higher mean IgG4 Ab level than the group with no subjective improvement ($P < 0.01$). However, this study is difficult to interpret for three reasons. Firstly, about half (12/23) of the patients were children [17]. As discussed [12] children may respond differently from adults to IT and the natural course of their allergic disease may be different from that of adults. Furthermore, the children were given rush IT with aqueous extracts during the dose-increase phase, whereas the adults were given single injections weekly with alum-adsorbed extracts during this phase. The children

developed higher IgG4 Ab levels than the adults and apparently behaved immunologically differently from adults in several aspects [9]. As high mean IgG4 Ab level, thus, was associated with classification both as 'child' and as 'improved', one might suspect that most of the improved patients were children, but no data were given to elucidate this aspect. If this actually was the case, the association between high IgG4 Ab level and good effect might have been due to a more favourable course of IT in children. Secondly, the amount of IgG4 Ab was not measured directly in diluted serum, as in all the other studies cited, but in isolated antigen-specific antibodies. The antigen-specific Abs were isolated by elution at pH 2.7 from column-packed allergosorbents. This approach carries the risk of losing high affinity Abs, which may be retained on the allergosorbent column. Thus, we (Djurup, unpubl. results) have found that only 20% of IgG4 Ab bound to a grass pollen column could be eluted, even by stepwise pH changes going as far down as to pH 2.0. As high affinity Abs may add appreciably to the signal in solid-phase assays [18], use of affinity-isolated Abs may seriously underestimate the amount of IgG4 Abs in sera from patients with high affinity IgG4 Abs. Thirdly, a very high number of significance tests were performed and some of the 'significant' outcomes might have been due to chance alone.

The study by Devey *et al.* [1] was concerned with short-term IT (2–4 months) and quantitation of IgG4 Abs was based on a semi-quantitative assay. They found no differences in the mean titre of IgG4 Ab between ten patients with good clinical response and nine patients with poor clinical response. However, when they looked separately on ten patients receiving IT with grass pollen adsorbed to tyrosine crystals, they found that the mean IgG4 Ab level shortly after the end of IT was significantly higher ($P < 0.05$) in the five patients who improved. However, they also found a significant association between decrease in mean titre of IgG2 Abs and clinical improvement in this sub-group.

We want to emphasize that we have only shown that the treatment failures are to be found in the group with late high IgG4 Ab level (and vice versa that almost any patient with low level improves). As about half of the patients with high level improved clinically we do not exclude the possibility that the association between high mean IgG4 Ab level and clinical improvement found in the studies by Nakagawa [8] and Hedlin *et al.* [9] may be real. However, in these studies no attention was paid to the possibility that a few patients with very high IgG4 Ab levels failed to improve or even became worse.

It should be noted that the relationship between high IgG4 Ab level and clinical result may stand more clearly out when analysed in 2×2 contingency tables (i.e. by using nominal classification) than when analysed on basis of ordinal or interval classification [4,7].

The joint statistical analysis as a scientific format

Performance of a joint statistical analysis of data from several trials requires that all trials are comparable and independent, and that all trials of the type in question—or a random sample thereof—are included in the analysis [10]. We think our analysis fulfils these criteria. Thus, we have excluded three non-comparable trials [1,8,9] and do not know of any other published trials on this topic. Due to the considerable interest among allergologists as to whether IgG4 Abs act as blocking or as sensitizing Abs (or both) we consider it unlikely that studies in favour of either role should have remained

unpublished. The strong association found in the joint analysis is, therefore, probably not due to 'publication bias' [10].

Joint statistical analyses have yielded important knowledge within other fields of internal medicine and may be considered an alternative to or a 'forerunner' of multi-centre trials [10]. As pre-requisites for doing joint analysis are that either data on the individual patient or exact probabilities are given, authors of papers on clinical trials within allergology should state at least the exact probabilities found.

Early IgG4 Ab level as predictor of final clinical outcome

The results of the statistical analysis of the data combined from studies II and III (Table 7) strengthen the original suggestion in study II [7] that early IgG4 Ab level may predict the clinical condition at the end of IT.

The time for measurement of 'early IgG4 Ab level' may be critical. At present we cannot give any rule for selection of this time which, therefore, has to be defined by retrospective studies (see below). However, it is obvious that selection of a too early time may make differentiation of intensity of IgG4 Ab production difficult due to lack of assay sensitivity and of assay precision near the detection limit. In contrast, later in the course the association between IgG4 Ab levels and final clinical outcome may weaken and the predictive value may become lower. Thus, the analysis of the combined data from studies II and III showed that the association between early IgG4 Ab level and final outcome ($P = 1.05 \times 10^{-4}$; Table 7) was much stronger than the association between late level and clinical result ($P = 1.92 \times 10^{-2}$; data not shown). This may indicate that the patient's predisposition to produce high IgG4 Ab level early in the course of an immune response (i.e. at a minimal stimulus) is the determinant proper of late treatment failure. Aalberse *et al.* [3] found that most patients with hay fever due to grass pollen had low levels of IgG4 Abs before initiation of IT. If pre-treatment levels of IgG4 Abs could be measured precisely it would be of great interest to evaluate the association between pre-treatment levels and long-term treatment failure.

On the biological basis underlying the association between high IgG4 Ab level and treatment failure.

The data presented in this paper lend some support to the hypothesis that IgG4 Abs may be sensitizing in some patients (reviewed and discussed in [19–21]). As only nine of nineteen patients with high IgG4 Ab level at the end of IT failed to improve, or became worse, other factors than the absolute or relative concentration of IgG4 Abs may be important in determination of their sensitizing ability. Stanworth [21] has suggested that different subtypes (e.g. different allotypes or differently glycosylated IgG4 molecules) may have different sensitizing abilities. Another possibility that we want to propose, is that IgG4 receptors on basophils and mast cells in different individuals may occur at different densities or may have different affinities for IgG4. Such differences could explain the great inter-individual differences in histamine release from basophils *in vitro* on stimulation with anti-IgG4 Abs [22–25]. Conceivably, patients with a predisposition to produce IgG4 Abs on minimal stimuli might also have high density or high affinity of the IgG4 receptors, which could explain the predictive value of early IgG4 Ab level. In patients with both high levels of IgG4 Abs and high density or affinity of IgG4 receptors, IgG4 Abs formed during IT could act as sensitizing Abs. In patients with low density or affinity of IgG4 receptors, the IgG4 Abs formed during IT

might act as blocking Abs by preventing the offending allergen in reaching the effector-cell bound-IgE molecules.

Modern techniques for isolation of human basophils [26] and for study of interaction between Fc fragments of IgG and Fc γ receptors, even of low affinity [27], should permit characterization of density and affinity of the presumed human IgG4 receptor on basophils.

Clinical implications

We have made our suggestion that early IgG4 Ab level has predictive value for the final outcome of IT on two retrospective studies. Although this association is well validated from a statistical point of view, further studies are necessary before any routine clinical use can be made of such association. Thus, it will be necessary to determine the cut-off value for 'early high IgG4 Ab level' for each inhalant allergen separately in retrospective studies (i.e. with known long-term result of IT) comprising as many patients as possible. Also, cut-off values may need to be determined for different injection schedules with the same allergen. Based on these studies the predictive value of early high IgG4 Ab level should then be confirmed in prospective studies, in which the final outcome for each patient is predicted at the time when the early IgG4 Ab quantitation has been performed [28].

References

- 1 Devey ME, Wilson DV, Wheeler AW. The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* 1976; 6:227-36.
- 2 Giessen Mvd, Homan WI, Kernebeek Gv, Aalberse RC, Dieges PH. Subclass typing of IgG antibodies formed by grass pollen-allergic patients during immunotherapy. *Int Arch Allergy Appl Immunol* 1976; 50:625-40.
- 3 Aalberse RC, Dieges PH, Knul-Bretlova V, Vooren P, Aalbers M, Leeuwen JV. IgG4 as blocking antibody. *Clin Rev Allergy* 1983; 1:289-302.
- 4 Etievant M, Leluc B, Bouclier R, Henocq E. Immuno-enzymatic study of IgG subclasses specific for allergen in house dust immediate hypersensitivity. *Ann Allergy* 1979; 43:169-73.
- 5 Parish WE. Short-term anaphylactic IgG antibodies in human sera. *Lancet* 1970; ii:591-2.
- 6 Stanworth DR, Smith AK. Inhibition of reagin-mediated PCA reaction in baboons by human IgG4 subclass. *Clin Allergy* 1973; 1:37-41.
- 7 Djurup R, Østerballe O. IgG subclass antibody response in grass pollen-allergic patients undergoing specific immunotherapy. Prognostic value of serum IgG subclass antibody levels early in immunotherapy. *Allergy* 1984; 39:433-41.
- 8 Nakagawa T. IgG subclass changes in response to desensitisation. *Monogr Allergy* 1986; 19:253-61.
- 9 Hedlin G, Graff-Lonnevig V, Heilborn H *et al*. Immunotherapy with cat- and dog-dander extracts. II. *In vivo* and *in vitro* immunologic effects observed in a 1-year double-blind placebo study. *J Allergy Clin Immunol* 1986; 77:488-96.
- 10 Andersen B, Holm P. Problems with *P* Significance Testing in Medical Research. 1st edn. Basel: Editions Roche, 1984:63-82.
- 11 Malling HJ, Dreborg S, Weeke B. Diagnosis and immunotherapy of mould allergy. V. Clinical efficacy and side effects of immunotherapy with *Cladosporium herbarum*. *Allergy* 1986; 41:507-19.
- 12 Djurup R, Malling HJ, Søndergaard I, Weeke B. The IgE and IgG subclass antibody response in patients allergic to yellow jacket venom undergoing different regimens of venom immunotherapy. *J Allergy Clin Immunol* 1985; 76:46-55.
- 13 Sokal RR, Rohlf FJ. Biometry. 2nd edn. San Francisco: Freeman and Company, 1981:731-47.
- 14 Siegel S. Non-parametric Statistics for the Behavioral Sciences. Tokyo: McGraw-Hill Kogakusha, Ltd, 1956:212.
- 15 Armitage P. Statistical Methods in Medical Research. 1st edn. Oxford: Blackwell Scientific Publications, 1971:427-33.
- 16 Sheps SB, Schechter MT. The assessment of diagnostic tests. A survey of current medical research. *J Am Med Assoc* 1984; 252:2418-22.

- 17 Sundin B, Lilja G, Graff-Lonnevig V *et al*. Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double-blind study on patients with animal dander asthma. *J Allergy Clin Immunol* 1986; 77:478-87.
- 18 Péterfy F, Kuusela P, Mäkelä O. Affinity requirements for antibody assays mapped by monoclonal antibodies. *J Immunol* 1983; 130:1809-13.
- 19 Gwynn CM. Role of IgG4 in allergy: clinical significance. In: Reed CE, ed. *Proceedings of the XII International Congress of Allergology and Clinical Immunology*. St Louis: The C.V. Mosby Co., 1986;60-4.
- 20 Stanworth DR. Immunochemical aspects of human IgG4. *Clin Rev Allergy* 1983; 1:183-95.
- 21 Stanworth DR. The molecular pathology of IgG4. *Monogr Allergy* 1986; 19:227-40.
- 22 Fagan D, Slaughter CA, Capra JD, Sullivan TJ. Monoclonal antibodies to immunoglobulin G4 induce histamine release from human basophils *in vitro*. *J Allergy Clin Immunol* 1982; 70:399-404.
- 23 Nakagawa T, Weck ALd. Membrane receptors for IgG4 subclass on human basophils and mast cells. *Clin Rev Allergy* 1983; 1:197-206.
- 24 Aalberse RC, Zee JSvd. Immunoassay in allergic diseases with special reference to IgG antibodies. In: Errigo E, ed. *Proceedings of XII Congress of the European Academy of Allergology and Clinical Immunology*. Florence: OIC Medical Press, 1983:73-80.
- 25 Hindocha P, Wood CBS. Histamine release from human leucocytes by IgG4 subclass in the sera of allergic children. *Allergy* 1985; 40:523-8.
- 26 MacGlashan DW, Lichtenstein LM. The purification of human basophils. *J Immunol* 1980; 124:2519-21.
- 27 Kurlander R, Niedel J. Affinity labeling of the Fc receptor on human monocytes using bifunctional cross-linking agents. *J Immunol Methods* 1985; 78:247-58.
- 28 Wasson JH, Sox HC, Neff RK, Goldman L. Clinical prediction rules. Applications and methodological standards. *N Engl J Med* 1985; 313:793-9.

Addendum

The paper referred to as [Malling and Djurup (in prep.)] has been accepted for publication in *Allergy*:

Malling H-J, Djurup R. Diagnosis and immunotherapy of mould allergy. VII. IgG subclass response and relation to the clinical efficacy of immunotherapy with *Cladosporium*. *Allergy* 1987; in press.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.