

Clinical significance of IgG subclass antibodies to wheat flour antigens in bakers

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We measured the IgG subclass antibody levels to wheat flour in 42 bakers and 20 controls with an enzyme immunoassay. The levels of total IgG, IgG1 IgG2 and IgG4 antibodies were significantly higher in the bakers than in the unexposed controls. The presence of anti-wheat flour IgG subclass antibodies in the bakers was correlated with various clinical variables including IgE levels, duration of asthmatic or rhinitis symptoms, skin prick test response, peripheral blood eosinophil levels, bronchial histamine reactivity and responses to nasal challenge with wheat flour. The IgG subclass antibody levels of the total cohort of bakers did not correlate with any of the measured clinical variables. However, among men specific IgG4 and IgG1 antibody levels correlated negatively with total IgE levels and duration of rhinitis, respectively. We conclude that IgG and IgG subclass levels to wheat flour in bakers reflect exposure, but that it is not related to any specific clinical situation. The exact pathogenetic role of these antibodies in the development of occupational asthma and rhinitis is thus not clear.

Key words: ELISA; IgG subclasses; wheat flour allergy.

Accepted for publication 19 April 1990

The ability to produce an IgG antibody response of certain subclasses has important consequences to the immunological defence of the host against bacteria and viruses (4, 14, 17). In viral diseases, IgG1 and IgG3 responses dominate, whereas IgG2 may be the main IgG antibody response to bacterial polysaccharides, and IgG1 is predominantly produced against proteins. IgG4 responses appear after repeated antigen stimulation or hyperimmunization (2, 10) and specific antibody responses against a number of foods, pollens and venoms belong to the IgG4 subclass (2, 16, 23).

In allergic diseases, the so-called “blocking” antibodies have been suggested to belong to the IgG class (9, 13, 18). However, there are a number of studies in which no association has been found between a rise in IgG and the degree of clinical improvement (15, 22) and the

hypothesis that IgG, acting as a blocking antibody, forms the immunologic mechanism of immunotherapy has been questioned (20). Although an increase of, for instance IgG4 antibodies against inhaled antigens in allergic diseases has been observed (8, 23), no study has clearly shown that the presence of specific IgG4 antibodies correlates with any particular clinical situation.

We have previously shown that bakers have significantly higher titers of IgG subclass antibodies to wheat flour than non-exposed persons (25). The present study was undertaken to correlate the specific IgG subclass levels to the clinical situation of bakers, including the pattern and duration of symptoms, skin and nose responses to allergen provocation, IgE and eosinophil levels in blood and histamine hyperreactivity.

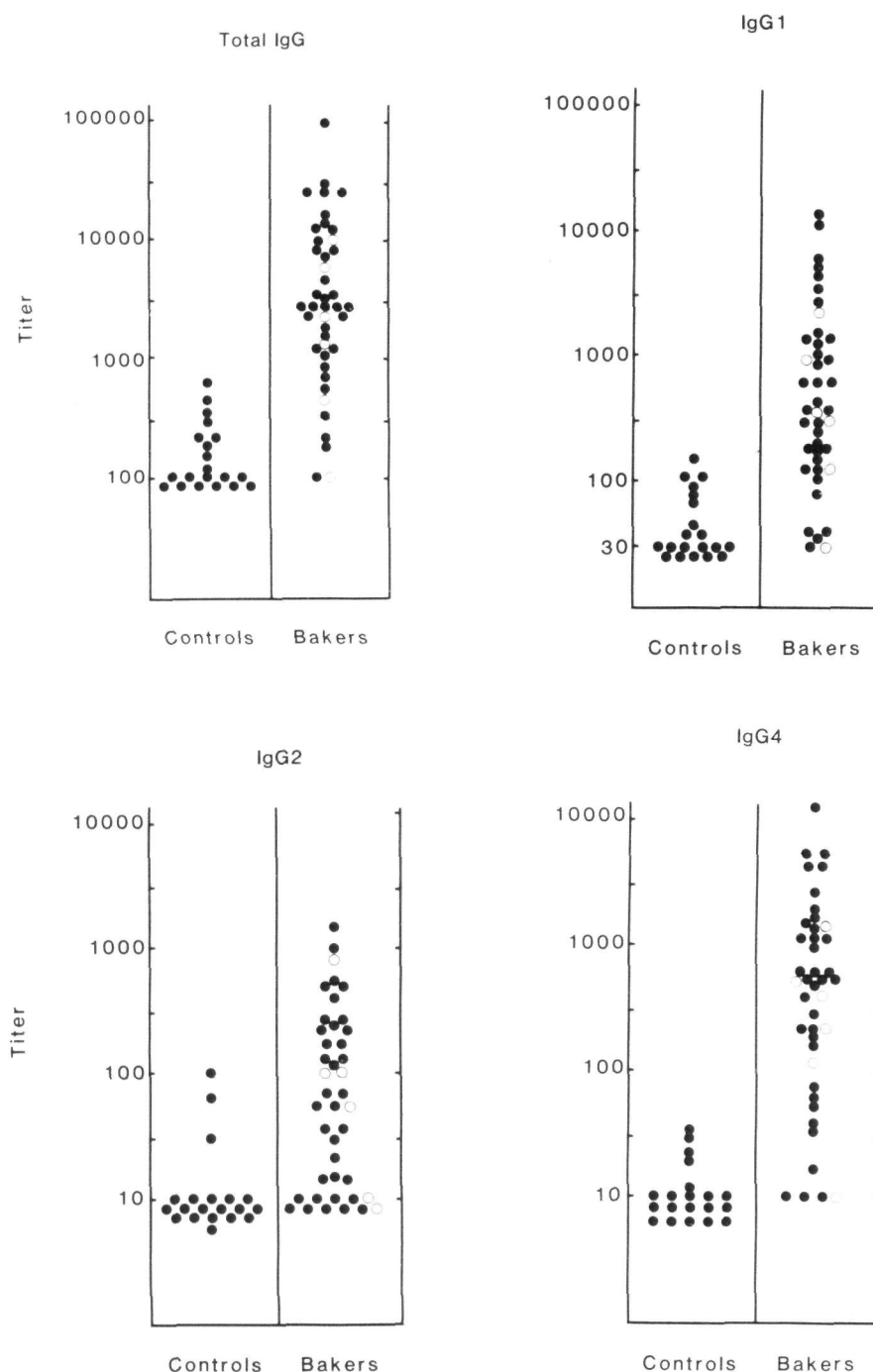


Fig. 1. Scattergrams showing the total and IgG subclass levels of anti-wheat flour antibodies in 42 bakers and 20 unexposed controls. (● wheat flour allergic bakers, ○ non-allergic wheat flour exposed bakers).

PATIENTS AND METHODS

Patients

Serum samples were obtained from 42 persons occupationally exposed to wheat flour, and suffering from work-related allergic symptoms. We studied 29 female bakers, median age 45 years

(range 21–61 years) and 13 males, median age 26 years (17–60 years). Control sera from non-exposed adults were obtained from eight women, median age 45 years (26–53) and 12 men, median age 46 years (33–60). The median exposure time was 11 years (range 2–32 years) for women and 9 years (1–31) for men. For both women and men the median duration of rhinitis symptoms was 4 years (range 0–18 years) and for asthmatic symptoms 2 years (0–18 years).

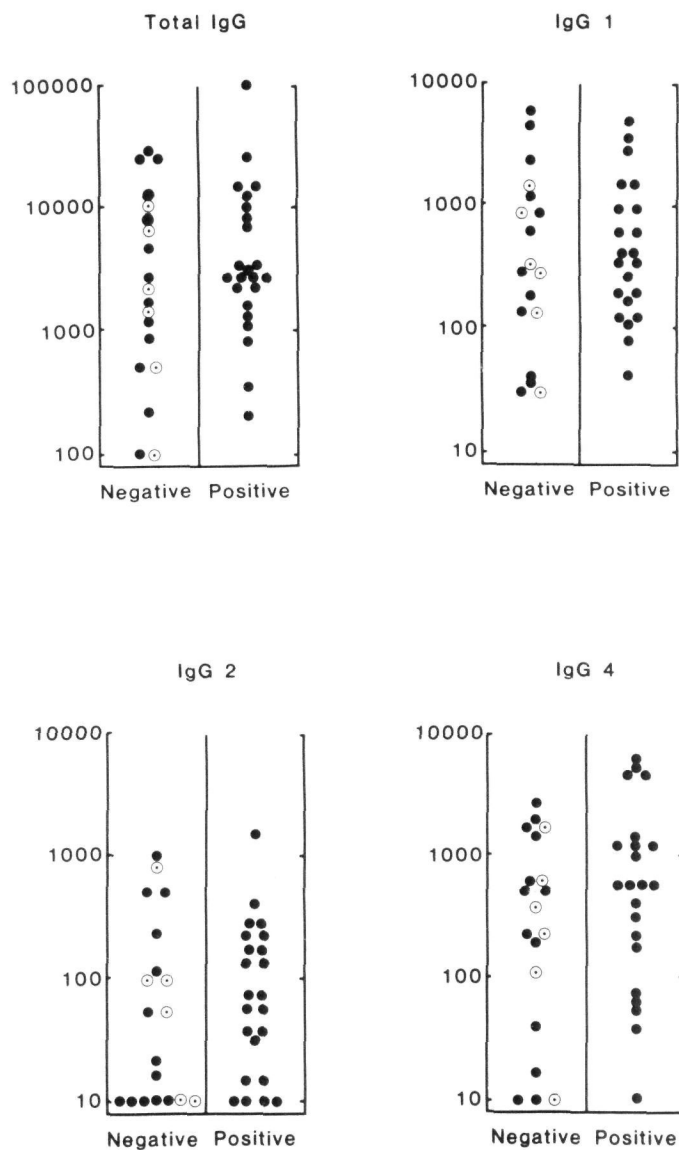


Fig. 2. Anti-wheat flour IgG subclass levels in relation to positive ($n = 23$) or negative ($n = 19$) skin reactivity (prick-test) to wheat flour. (● wheat flour exposed bakers, ○ non-allergic wheat flour exposed bakers).

Of the 42 bakers, 36 were considered to have specific wheat flour allergy based on the presence of typical symptomatology and a positive test result to wheat flour provocation of the skin, nasal mucosa or bronchi, or the presence of specific IgE against wheat flour. The other six patients (all women) were all exposed to flour and had work-related symptoms, but specific wheat flour allergy, as defined above, could not be detected.

Antigen and antisera

Wheat flour antigens were prepared as described earlier (6, 25). Monoclonal mouse anti-

human IgG subclass antibodies were purchased as ascites fluid from Oxoid, England (anti-human total IgG, clone 8a4, anti-human IgG1 NL16, anti-human IgG2 GOM 1, anti-human IgG4 RJ4). Alkaline phosphatase labelled rabbit anti-mouse IgG was obtained from Sigma, USA. The enzyme immunoassay (EIA) was carried out as previously described (25). Polystyrene microplate wells (Microstrips, Eflab, Finland) were coated overnight with 125 μ l of 5 μ g/ml wheat flour antigen in PBS for assays of total IgG, IgG1 and IgG4. In the IgG2 assay, the coating concentration was 100 μ g/ml. Serial dilutions of serum were incubated overnight. Anti-human IgG subclass antibodies were diluted 1:1000 for total IgG, IgG1, IgG4 and 1:500 for IgG2. Anti-mouse IgG-alkaline phosphatase conjugate (1:1000) was added, and after addition of substrate and the stopping of the reaction the absorbances were read at 405 nm using Titertek-Multiscan photometer, Flow Laboratories, England. The absorbances (A) measured were changed into arbitrary units (EIU) with the help of an in-house reference serum.

$$\text{EIU} = \frac{A(\text{sample}) - A(\text{blank})}{A(\text{ref}) - A(\text{blank})} \times 100$$

Results were expressed as the reciprocal dilution at which the serially diluted serum sample gave the value of 10 EIU. Selected positive control sera (one high positive, another low positive) were included in each run for each subclass. The coefficients of variation (CV) between days were 8–28% ($n = 11$) for high reference serum and 12–29% ($n = 11$) for low reference serum in different subclass assays. No IgG3-positive sera could be found.

The total IgE and the wheat flour specific IgE were measured using Phadezym Prist and Phadezym RAST assays, respectively (Pharmacia, Diagnostics, Sweden). In the RAST assay wheat flour positivity was defined as IgE levels above 0.35 kU/l.

Skin prick tests were performed according to the method of Aas & Belin (1). Antigens used were commercial freeze-dried wheat flour extract (Allergologisk Laboratorium, Copenhagen, Denmark) and non-commercial, parallel-tested ex-

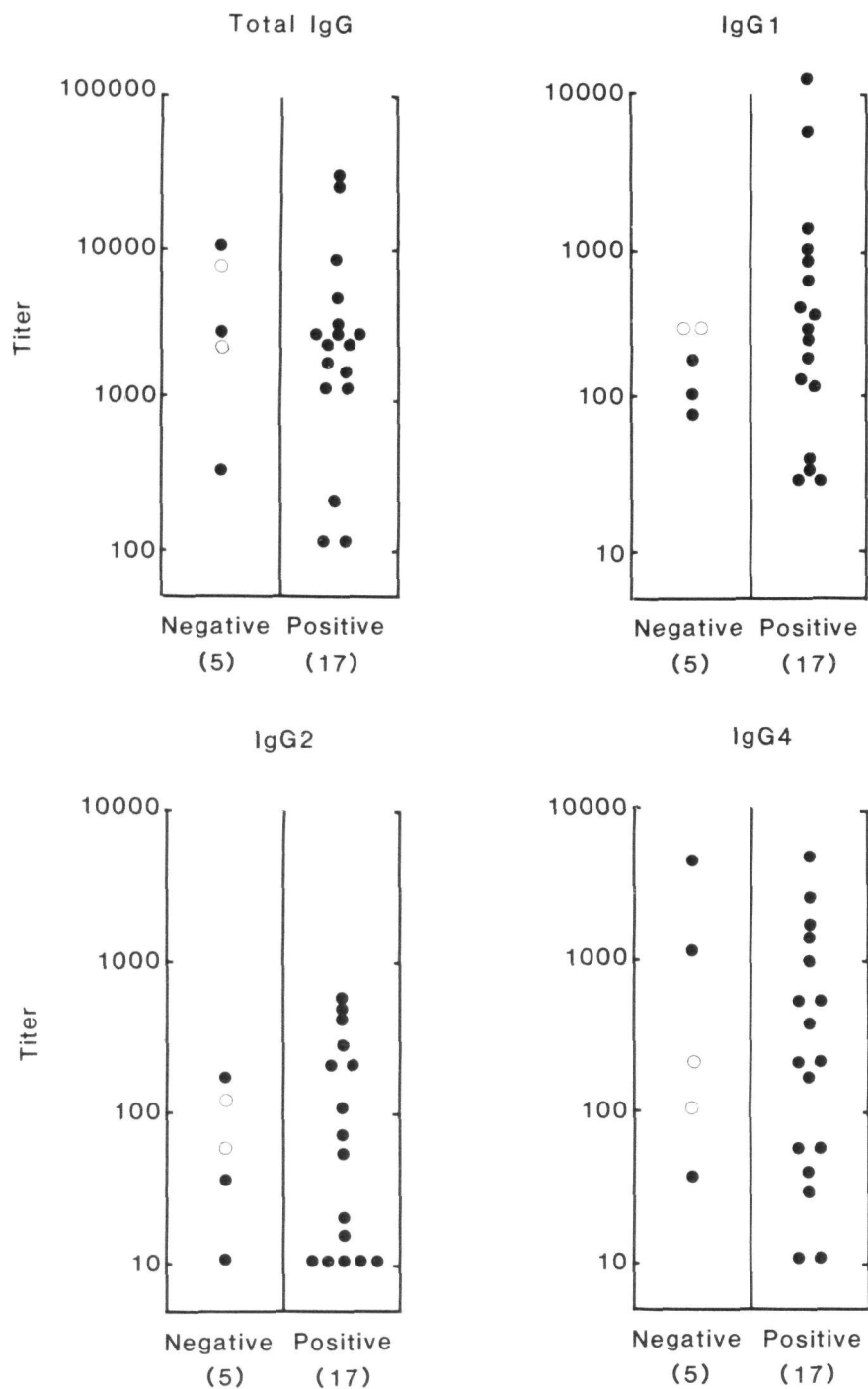


Fig. 3. Comparison of anti-wheat flour IgG subclass antibodies in patients with positive ($n = 17$) and negative ($n = 5$) response to wheat flour nasal provocation. Symbols as in Fig. 1.

tract of mixed wheat flour. Histamine (1 mg/ml or 10 mg/ml) was used as positive control. A positive wheat flour reaction was defined as a reaction $\geq$ half of the reaction to histamine (1 mg/ml).

Nasal and bronchial response to wheat flour was tested during dusting of wheat flour in a closed chamber. Dust levels were occasionally determined during the provocation tests and they were in the range of 10-100 mg/m³ (T.

Tuomi, personal communication). Nasal responses to flour provocation were evaluated by inspection and considered positive in the presence of rhinorrhea, swelling and sneezing.

Histamine hyperreactivity was determined according to Eiser et al. (11) and Cockcroft et al. (7). Eosinophils were counted in a Bürker chamber after staining for 10 min in 0.05% phloxine-propylene-glycol.

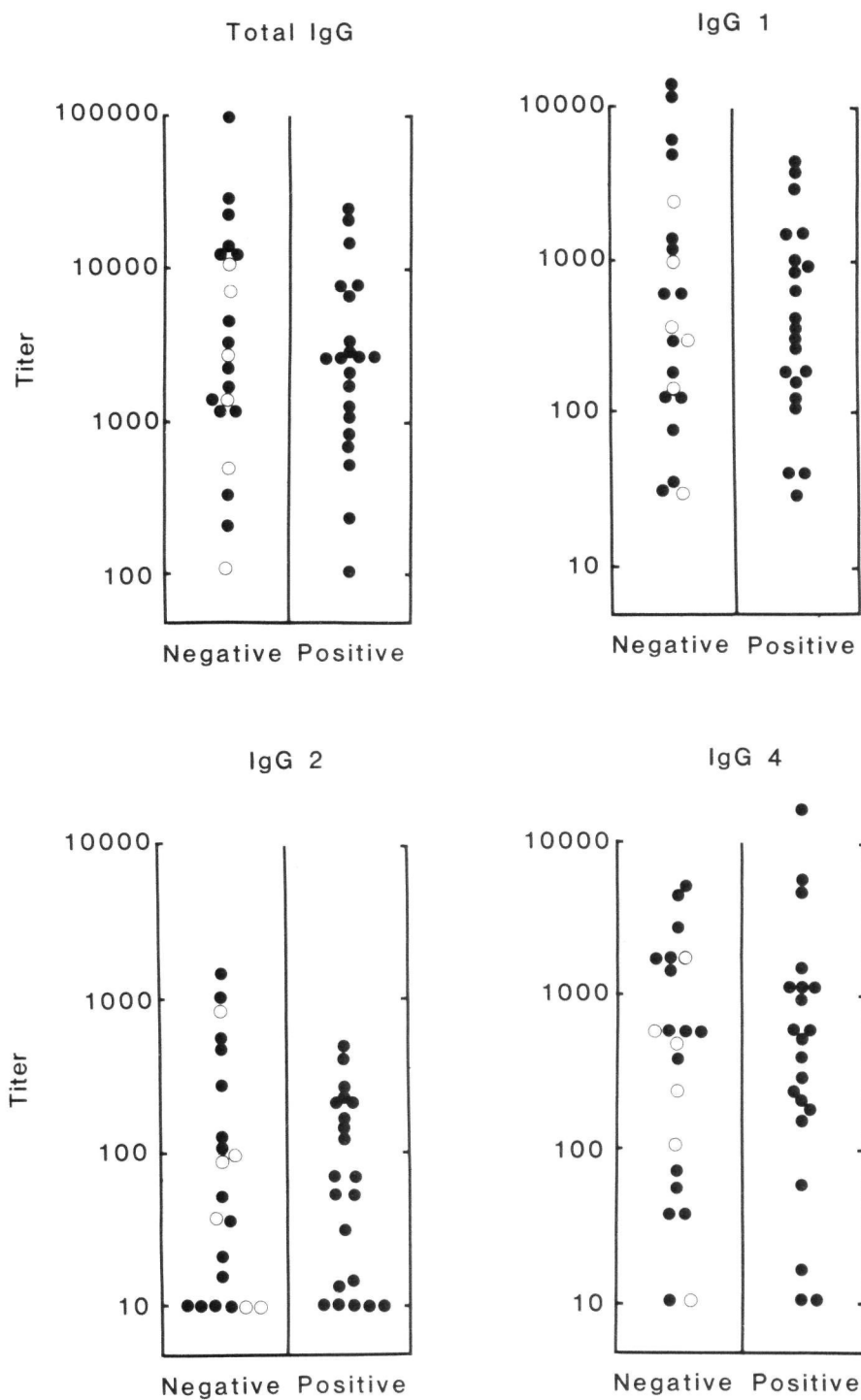


Fig.4. Anti-wheat flour IgG subclass levels in patients with the presence ($n = 21$) or absence ($n = 21$) of specific IgE antibodies. Symbols as in Fig.1.

Statistics

The χ^2 -test and the Wilcoxon rank sum test were used to assess the significance of differences between antigen-specific IgG subclass antibodies in bakers and controls.

RESULTS

The levels of total IgG, IgG1, IgG2 and IgG4 antibodies against wheat flour levels were significantly higher ($P < 0.001$) in the bakers than in the unexposed controls (Fig.1). Also six patients with wheat flour exposure but without specific wheat flour allergy (see Material and

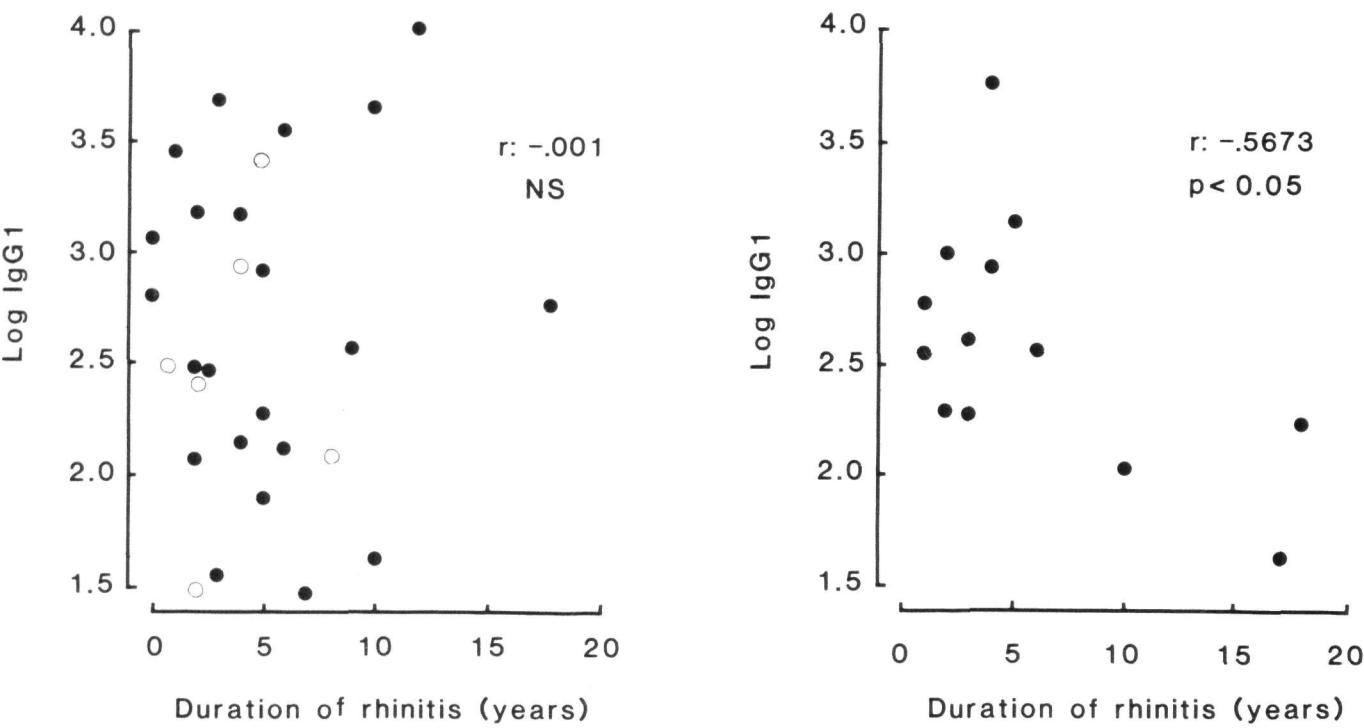


Fig. 5. Correlation between the anti-wheat flour IgG1 levels (log values) and duration of rhinitis in 29 female (left) and 13 male bakers (right). Symbols as in Fig. 1.

methods) had significantly ($P < 0.01$) higher antibody titers than the controls. No specific IgG3 antibodies could be detected. In women bakers, the occurrence of different IgG subclass antibodies was strongly associated ($P < 0.01$) between total IgG antibodies and IgG1, IgG2 and IgG4 levels and between subclasses separately. In men the subclass levels correlated with total IgG levels but not with each other.

No correlation could be demonstrated between the presence of specific IgG subclass antibodies and skin reactivity to wheat flour (Fig. 2). Neither did the response in the nasal provocation to wheat flour correlate with the titers of IgG antibodies (Fig. 3); there was no difference between women and men in these respects. Neither was there any correlation between IgG subclass titers and response to bron-

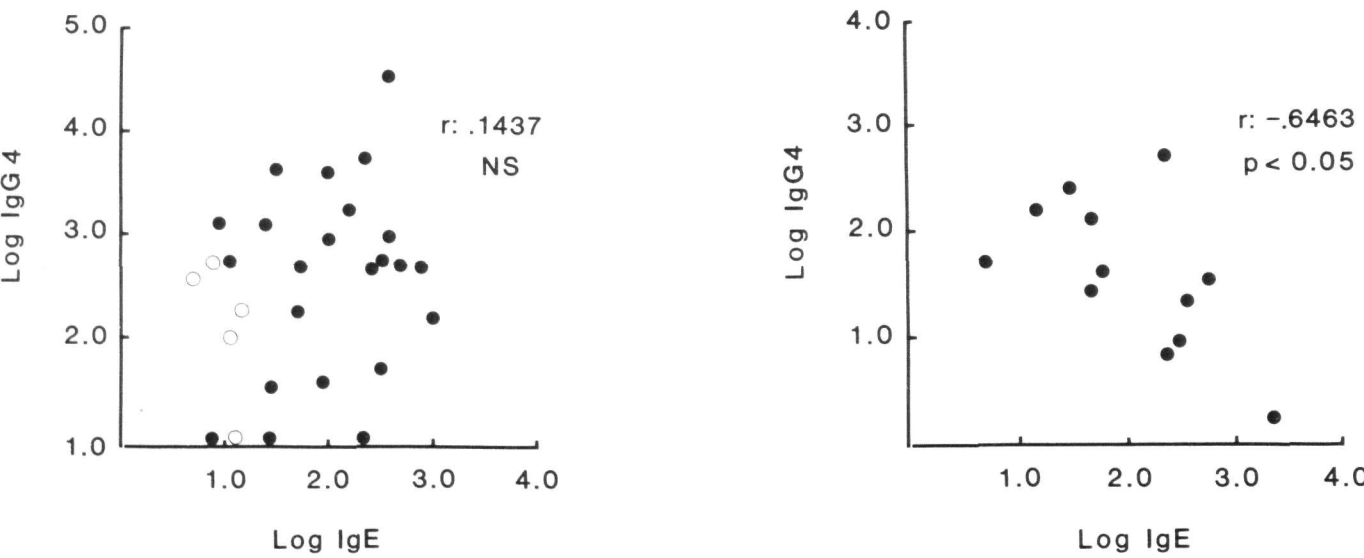


Fig. 6. Correlation of total IgE antibodies and wheat flour specific IgG4 antibodies in 29 female and 13 male bakers. Symbols as in Fig. 1.

chial provocation tests to wheat flour (data not shown).

Fig. 4 shows the IgG subclass titers to wheat flour in relation to their specific IgE levels in serum. No difference between RAST-positive and RAST-negative bakers could be observed, and there were no sex-associated differences in IgG levels.

We found a significant negative correlation between the titer of anti-flour IgG1 subclass antibodies and duration of rhinitis symptoms among male bakers ($n = 13$, $r = -0.5673$, $P < 0.05$) (Fig. 5), whereas no correlation was seen among female bakers. The levels of anti-wheat flour IgG4 correlated negatively with total IgE levels among men ($n = 12$, $r = -0.6463$, $P < 0.05$), whereas no correlation was seen among women (Fig. 6). Further, a significant positive correlation was observed between the IgG1 subclass titer and total number of eosinophils in the peripheral blood in women ($n = 27$, $r = 0.3975$, $P < 0.05$).

DISCUSSION

In this study we examined the distribution of IgG subclass anti-wheat flour antibodies in 42 bakers with wheat flour exposure and work-related symptoms.

Antibodies to wheat flour belonging to different IgG subclasses were detected in higher titers in both wheat flour-allergic and non-allergic but exposed bakers, compared with non-exposed subjects. However, we did not find a clear relation between the IgG subclass levels and the clinical expression of symptoms, response to nasal or bronchial exposure or other disease manifestations among these bakers.

There was no relation between the IgG subclass titer and the individual responses to wheat flour skin test, or the presence of specific IgE antibodies. These results are in concordance with studies showing a lack of correlation between specific IgG antibodies and any particular clinical situation (2, 24). Also for laboratory animal allergens, it has been reported that 50% of exposed individuals had detectable IgG antibodies to animal proteins with no correlation to the clinical picture (5). Our results are in agreement with studies suggesting that the presence

of IgG antibodies is more related to exposure than to the development of allergy. This lack of correlation between IgG subclass levels and clinical findings may partially be explained by the presence of "non-allergenic" proteins in our wheat flour extract, and it is likely that IgG antibodies will also be produced and measured to these proteins. It is also possible that the IgG subclass responses may be individually genetically restricted.

Our results show sex-related differences in the IgG subclass responses in relation to some variables associated with allergic manifestations. The negative correlations between anti-wheat flour IgG1 levels and duration of rhinitis observed among men in our survey, may be analogous to the changes that occur during immunotherapy. Several studies have demonstrated that a marked rise in the serum level of antigen-specific IgG is seen early during immunotherapy and a decrease in serum antigen-specific IgE occurs after long-term immunotherapy (10). In our study the highest IgG1 levels were found in bakers with short exposure, i.e., short duration of rhinitis symptoms, after which the IgG1 levels decreased in bakers with a longer duration of nasal symptoms. Although we could not find any significant changes in IgG4 in relation to exposure time or duration of symptoms, we observed an inverse relationship between IgG4 antibodies and total IgE levels among men. It is possible that antigen-specific IgG antibodies may be beneficial by inducing suppression of specific IgE antibody production (19), but the present cross-sectional study on bakers does not give significant evidence for this attractive hypothesis. Why such interrelationships should occur only among men, as is suggested by our study, remains to be explained. It is, however, known that there are sex-related differences in IgG response, and particularly the total IgG4 levels have been reported to be higher in men than in women (3, 12, 21).

We conclude that the development of IgG subclass antibodies depends particularly on antigen exposure, but the role of these antibodies in the pathogenesis of environmentally induced allergy remains uncertain.

ACKNOWLEDGEMENTS

We thank Drs L. Kanerva, O. Korhonen, H. Nordman and E. Vaheri for stimulating discussions and Ms L. Halmepuro, M.Sc., for her interest and help throughout this study. The excellent technical assistance of Ms T. Mäkelä is acknowledged.

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