

The primary immune response in bronchial asthma

I. A kinetic study of *Helix pomatia* hemocyanin-specific IgE, IgG, IgA, and IgM antibody responses in patients with asthma and in matched controls

F. R. Weller,* C. G. M. Kallenberg, M.D.,* H. M. Jansen, M.D.,***
R. Torensma, Ph.D.,* R. J. L. Klaassen,* H. H. Weller, M.D.,****
N. G. M. Orie, M.D.,** and T. H. The, M.D.*

Groningen, Amsterdam, and Haarlem, The Netherlands

Eleven asthmatic patients with allergy and 10 age- and sex-matched controls were immunized subcutaneously with 1 mg of the primary test immunogen Helix pomatia hemocyanin (HPH). The HPH-specific IgE, IgG, IgA, and IgM antibody response was measured by ELISA. After immunization, not only the IgE but also the IgG antibody response of the patients exceeded that of the controls ($p < 0.01$). IgA antibody response also tended to be higher in the asthmatic group (NS). Except for an earlier rise after immunization in the asthmatic group ($p < 0.05$), IgM peak responses did not differ significantly between the two groups. There was a high correlation ($r = 0.9$) between the magnitude of the IgE and the IgG antibody responses. IgM antibody response did not correlate with the response in any of the other antibody classes measured. The total serum immunoglobulin concentration was determined before immunization. Only the IgE level was significantly higher in the asthmatic group ($p < 0.01$). No correlation was found between serum immunoglobulin concentrations and the magnitude of the HPH-specific antibody response in the same class. We conclude that the increased humoral responsiveness of asthmatic patients with allergy is not restricted to the IgE class nor to a limited number of commonly encountered antigens (allergens). (J ALLERGY CLIN IMMUNOL 76:29-34, 1985.)

Young patients with asthma often have elevated serum levels of allergen-specific IgE antibodies. As the role of IgE in asthmatic manifestations is well established, much interest has been focused on the mechanism responsible for the increased generation of IgE antibodies in asthma. The induction and study of a primary immune response offers a good test system in which to collect information about the regulation of antigen-specific antibody responses. By using primary antigens one can avoid the effect of differences

Abbreviations used

HPH: Helix pomatia hemocyanin
ELISA: Enzyme-linked immunosorbent assay
BSA: Bovine serum albumin
KLH: Keyhole limpet hemocyanin

From the Departments of Clinical Immunology,* and Pulmonology,** University Hospital Groningen, Department of Pulmonology,*** University of Amsterdam, and St. Elisabeth of Groote Gasthuis,**** Haarlem, The Netherlands.

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Reprint requests: F. R. Weller, Department of Clinical Immunology, University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

in antigen-specific immunologic memory between the test subjects on the responses after exposure. Parenteral administration of the antigen allows standardization of antigen exposure to the immunocompetent cells. Moreover, evaluation of the responses of patients and controls to a test antigen might indicate whether immunoregulatory abnormalities are restricted to the responses directed against a relatively small number of well-known allergens or whether they also apply to a randomly chosen primary antigen.

IgE antibody response may be regulated separately from the response in the other antibody classes.¹ In some studies, however, elevation of allergen-specific

IgE antibodies in patients with asthma was reported to coincide with high levels of IgG antibodies with the same specificity.² To compare immunoregulatory aspects of the IgE response with those in the other classes, antigen-specific IgG, IgA, IgM, and IgE antibody levels should be studied simultaneously after immunization.

In our study a primary immune response was induced in 11 patients with asthma and 10 sex- and age-matched healthy controls by parenteral administration of the test immunogen HPH. Earlier experience with HPH in our laboratory has shown that this primary, T cell-dependent antigen offers a safe and well standardized tool to induce an immune response in virtually all healthy subjects.³ For the first time, IgE antibody responses of patients with asthma are described after primary immunization. In addition, HPH-specific IgE, IgG, IgA, and IgM antibody responses were recorded kinetically in both patients and controls. Responses of both groups were compared to evaluate the class specificity and the antigen restriction of the humoral hyperresponsiveness in asthma.

MATERIAL AND METHODS

Subjects

Ten normal control subjects and 11 patients with asthma were studied. All were male students between 19 and 28 years old (median age of patients was 22 years and that of the controls was 23 years) and all gave fully informed consent. Patients with relatively mild symptoms were selected so that results would not be biased by the stress of asthmatic attacks. None of the patients used theophylline or sympathomimetic drugs. Medication with oral steroids had been excluded for at least 1 year before the test.

Routine intracutaneous tests were done with house dust mite, pollen, molds, and animal danders (Diephuis Laboratories, Groningen, The Netherlands). All patients showed a positive reaction (diameter >5 mm) to at least one allergen and all had increased aspecific bronchial reactivity as assessed after bronchial histamine provocations (reduction of the forced expiratory volume in 1 second of 20% at histamine concentrations <32 mg/ml).⁴

The control subjects had no positive skin reactions or positive bronchial provocation reactions at concentrations <32 mg/ml. Severe infections or surgical interventions were excluded by history for a period of at least 3 months before the test. Routine laboratory test results excluded intercurrent infections and liver, renal, or endocrine disturbances. More detailed information about our subjects will be provided elsewhere.⁵

Immunization procedure

All subjects were immunized at the same time of the day with 1.0 mg subcutaneous HPH in the deltoid region as described.^{3,6} Blood was drawn before and 7, 14, 21, and 32 days after immunization to study the kinetics of the humoral immune response in patients with asthma and controls.

Detection of class-specific antibodies to HPH

IgE, IgA, IgG, and IgM antibodies to HPH were determined by an indirect ELISA technique.³ Briefly, flat-bottom microtiter plates (M 129 A; Dynatech R/D Co., Cambridge, Mass.) were coated with HPH in 0.1M sodium carbonate at pH 9.6 (5 µg HPH per milliliter) for at least 2 days at 4° C. After washing three times with 10mM Tris/HCl (the latter contained 0.15M NaCl and 0.05% Tween-20), the plates were incubated for 45 minutes at 37° C with 100 µl serum dilutions (1:100 to 1:1600) in 10mM Tris/HCl at pH 8.0 containing 0.3M NaCl, 0.05% Tween-20, and 4% BSA.

After washing the plates were incubated for another 45 minutes with 1:1000 diluted heavy chain-specific antisera (rabbit antihuman) conjugated to horse radish peroxidase (Pasteur, Paris, France) in 10mM Tris/HCl at pH 8.0 containing 0.3M NaCl, 0.05% Tween-20, and 2% BSA. After washing bound enzyme activity was measured by incubation with 100 µl 50mM phosphate buffer at pH 5.6 containing 0.2 mg/ml orthophenylene-diamine and 0.0045% H₂O₂ for 30 minutes at room temperature. The reaction was stopped by the addition of 100 µl 1N H₂SO₄. Optical density at 492 nm was read with a Titertek multiscan (Flow Laboratories, McLean, Va.) and the values were entered into a computer for subsequent calculations. Each plate contained blank incubations and a reference sample assayed in duplicate at six concentrations. The reference sample consisted of a pool drawn from 20 high responders 3 weeks after the primary HPH immunization.

Anti-HPH concentrations were computed from the linearized titration curve obtained after log-logit transformation of the concentrations in the reference serum and the corresponding optical densities. Antibody concentrations were expressed in arbitrary units as a percentage of the reference sample. Antibody concentrations had to be covered by the titration curve. A double-layer technique was used to determine IgE antibodies. Instead of enzyme-conjugated anti-human heavy chain antibody, unconjugated rabbit anti-human IgE was used. After washing, peroxidase-conjugated goat anti-rabbit Ig was added. Further steps are identical to the procedure described above.

The rabbit anti-human IgE was from the Central Laboratory of the Blood Transfusion Service in Amsterdam. Specificity of rabbit anti-human IgE had been tested by passive hemagglutination. In addition, contaminating IgG antibodies were excluded by the addition of excess human IgG to the ELISA system. Whereas the excess IgG significantly lowered the levels of HPH-specific IgG antibodies, there was no effect of excess IgG on the measurement of IgE antibodies. Moreover, IgG-producing Kahler myeloma cells did not react with the anti-IgE we used.

Detection of serum immunoglobulin levels

IgG, IgM, and IgA serum concentrations were determined with nephelometric techniques. IgE serum concentrations were measured by radioimmunoassay (Phadebas IgE test; Pharmacia Diagnostics, Piscataway, N. J.).

Statistics

Wilcoxon's rank sum test was used for statistical analysis of differences between the two groups. Spearman's rank

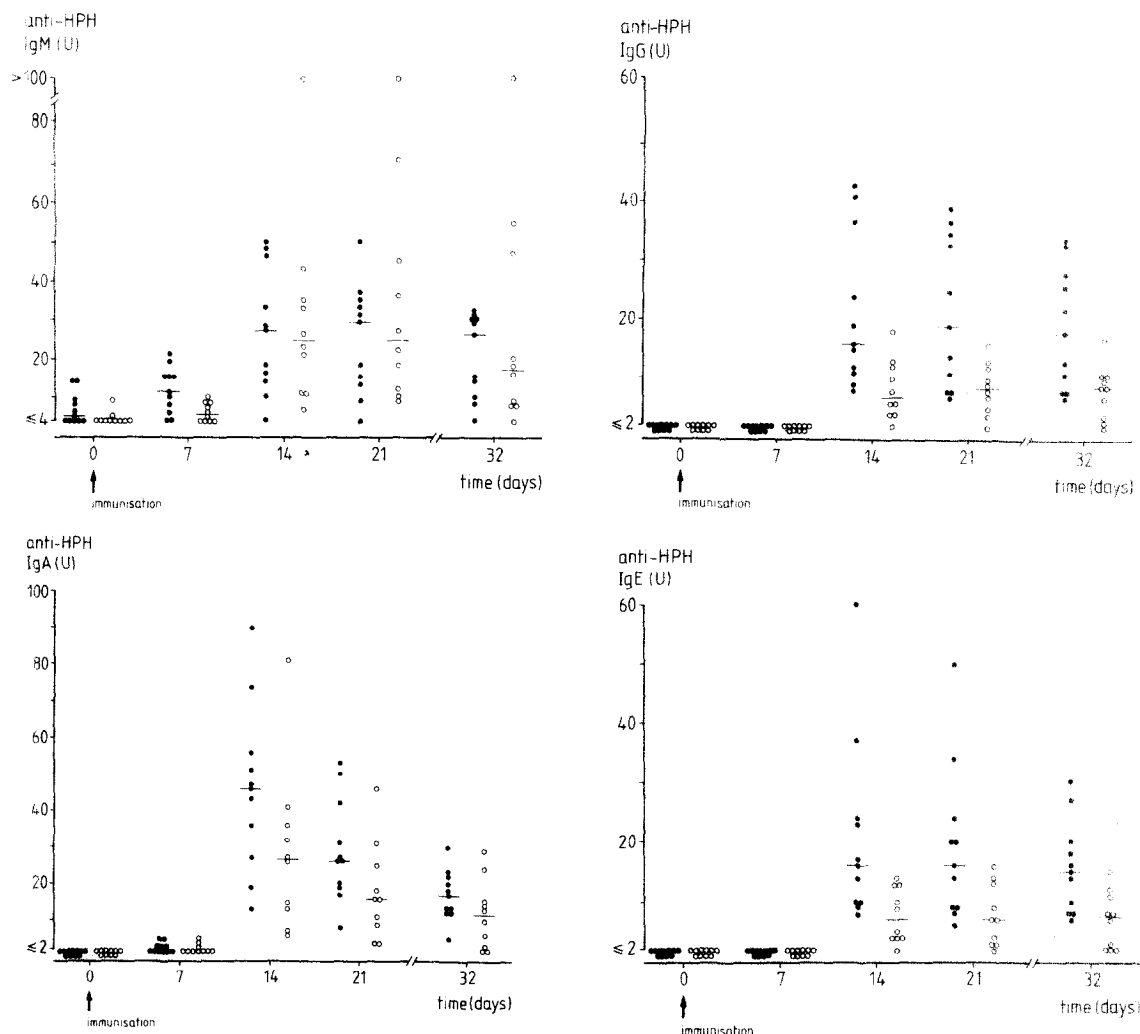


FIG. 1. HPH-specific antibody concentrations (percentage of reference serum value) plotted against time (in days) after immunization with HPH, 1.0 mg subcutaneously in patients with asthma (●) and in healthy controls (○). Horizontal bars represent the median value of the group.

correlation coefficients were computed. P values <0.05 were considered significant.

RESULTS

HPH-specific antibody responses

The 11 patients with asthma and the 10 sex- and age-matched controls were injected subcutaneously with 1.0 mg HPH (Fig. 1; Table I). HPH-specific antibody responses were recorded kinetically for 32 days.

Before immunization, only IgM antibodies (levels >4 U) were detected in seven patients and four controls. This is probably because of cross-reaction with the carbohydrate moiety of the HPH molecule,⁷ in accordance with earlier observations.³ The median value was 5 units for the asthmatic group (range 1 to 14 units) and 3 units for the control group (range 0 to 9 units). After immunization, an increase in the IgM antibody level was already evident at day 7 in

both groups. At that time the response of the patients was greater than that of the controls ($p < 0.05$). The peak IgM antibody level, which was reached between days 14 to 21, did not differ between the patients and the controls. At day 32 the IgM antibody level had declined in both groups. There was no correlation between the preimmune serum level of IgM and the peak antibody response in the IgM class.

At day 7 after immunization, IgA antibody was recorded in only seven patients and four controls. The peak level was reached at day 14 in both the patients (median value 46 units; range 13 to 90 units) and the controls (median value 27 units; range 6 to 81 units). At day 21 the IgA antibody level of the patients and the controls had already declined and at day 32 further decrease in IgA antibody level became apparent. Although the IgA response in the patients tended to be higher than that in the controls, there was no significant difference between the IgA antibody response

TABLE I. Class-specific antibody responses of 11 patients with asthma and 10 matched controls after immunization with the primary test immunogen *Helix pomatia* hemocyanin

Time after immunization (days)	IgM class anti-HPH (units)		IgA class anti-HPH (units)		IgG class anti-HPH (units)		IgE class anti-HPH (units)	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
0	5 (1-14)	<4	<2	<2	<2	<2	<2	<2
7	11 (2-21)	6* (3-10)	2 (0-5)	<2	<2	<2	<2	<2
14	27 (4-50)	25 (7-100)	46 (13-90)	27 (6-81)	16 (8-43)	7† (2-18)	16 (8-60)	7† (4-14)
21	29 (4-50)	25 (9-100)	26 (8-53)	16 (4-16)	19 (6-50)	9* (2-16)	16 (7-39)	7* (2-16)
32	26 (4-32)	17 (0-100)	17 (5-30)	12 (0-29)	18 (7-34)	9* (2-17)	15 (7-30)	8† (0-15)

Data are median (range).

* $p < 0.05$; † $p < 0.01$.

of the groups. No correlation was observed between the peak response of IgM and IgA antibodies in both groups.

In contrast to the early rise in IgM and IgA antibody levels, IgG antibodies were not detectable at day 7 in the control group. In the patient group, however, three showed positive results (≥ 2 units). A sharp rise in both groups between days 7 to 14 resulted in a peak level at day 14 in the patients (median value 16 units; range 8 to 43 units) and in the controls (median value 7 units; range 2 to 18 units). There was no change in level in the control group at days 21 and 32, but there was a significant decline in the patient group between days 21 and 32. The two groups differed significantly in IgG antibody level at day 14 ($p < 0.01$), day 21 ($p < 0.05$), and day 32 ($p < 0.05$). At all these time points the response of the patients exceeded that of the controls.

There was a close correlation between the peak response of IgG and IgA antibodies only in the control group ($r = 0.82$; $p < 0.01$), but no correlation was found between the peak response of IgG and IgM antibodies in either the patient group or the control group. Kinetics of the IgE antibody response closely paralleled those of the IgG response. Both the relatively early rise of the response at day 7 in two of the patients without a detectable response in the controls and the sharp increase in antibody level at day 14 resembled the IgG response pattern. The peak response occurred at day 14 both in the patients (median value 16 units; range 8 to 60 units) and the controls (median value 7 units; range 4 to 14 units). Those levels persisted at days 14 and 21.

At day 32 only the patient group showed a signif-

icant decrease in the IgE antibody level, in parallel with the IgG response pattern. Differences in IgE antibody level between the patients and the controls were significant at day 14 ($p < 0.01$), day 21 ($p < 0.05$), and day 32 ($p < 0.01$). At all these time points the patients had a higher HPH-specific IgE antibody level than the controls.

There was excellent correlation between the peak level of IgE and IgG antibodies in both groups (patients: $r = 0.88$, $p < 0.001$; controls: $r = 0.93$, $p < 0.001$). The peak level of IgE and IgA antibodies correlated in the control group ($r = 0.82$; $p < 0.01$) but not in the patient group. There was no correlation between the IgE and IgM antibody response in either patients or controls.

Total serum immunoglobulin concentrations

Serum Ig concentrations had been measured before immunization in all subjects. There was no significant difference between patients and controls in the concentrations of serum IgM, IgA, or IgG. Serum IgE levels, however, were significantly ($p < 0.01$) higher in the patients (median value 396 U/L) than in the controls (median value 76 U/L). There were no correlations between the serum levels and the peak antibody responses in the respective Ig classes after immunization.

DISCUSSION

Immunologic processes are believed to be important in the pathogenesis of bronchial asthma. Young adults with asthma often have high levels of IgE antibodies directed against environmental allergens. Bronchial challenge with those allergens often causes exacer-

bations of dyspnea in the presence of allergen-specific IgE antibodies.⁸

The patients with asthma in our study were characterized by high serum IgE levels. The levels of IgG, IgA, and IgM, however, did not differ from those measured in the control group. Animal experiments have indicated that the IgE response may be induced and regulated separately from the response in the other antibody classes.¹ An appreciable amount of IgE in patients with asthma is induced by and directed against a small group of common allergens.² It is thus suggested that class-specific dysregulation of the IgE response to a limited number of common allergens may contribute to this immunologic difference between patients with asthma and healthy controls. However, when the immune system of the subjects in our study was stimulated with a primary antigen, antibody responses not only of IgE but also of the IgG class of the patients exceeded those of the controls. This suggests a more diffuse humoral hyperresponsiveness in patients with asthma. The involvement of IgA antibody hyperresponsiveness in patients with asthma is not supported by our results, as there was no significant difference between patients and controls (although levels tended to be higher in the patients). A mechanism operative during the immune response that is responsible for the limitation of the antibody response within physiologic borders apparently does not function adequately in patients with asthma. The actual localization of this dysregulation remains to be elucidated. Genetic factors that determine immune regulation might be involved.⁹ Because IgE as well as IgG antibody response is under T cell control, evaluation of T-suppressor cell function might provide useful additional information. Indeed, mononuclear cells of normal controls were reported to suppress IgE production *in vitro* by lymphocytes of atopic donors, whereas suppression of IgM and IgG antibody production under the same conditions was less.¹⁰ Other studies, however, revealed no difference between patients with asthma and controls in concanavalin A-induced suppressor cells affecting Pokeweed mitogen-driven IgG¹¹ and IgE¹² synthesis.

The observation that elevations in allergen-specific IgE levels in patients with asthma is accompanied by increased specific IgG levels has been described in conditions of natural exposure.² However, comparative studies of the primary immune response in patients with asthma and controls are relatively scarce. Salvaggio et al.¹³ immunized patients with rhinitis and controls with the primary immunogen KLH. Wheal and flare skin reaction on intradermal challenge with KLH induced 2 months after immunization appeared more frequently in the patient group. Hemaggluti-

nating antibody responses, however, were similar in both groups. Since antibodies assessed by hemagglutination mainly belong to the IgM class,¹⁴ this finding is in agreement with our observation of a similar IgM response in patients and controls. IgE antibody response of children with asthma after a booster injection with diphtheria and tetanus toxoid was reported to exceed that of age-matched controls.¹⁵ These observations provide further evidence that hyperresponsiveness of the IgE antibody response in patients with asthma occurs not only after exposure to a restricted group of normally occurring allergens but also after parenteral immunization with other antigens.

The excellent correlation between IgE and IgG antibody response after immunization with HPH in patients as well as controls suggests parallel immunoregulatory pathways. The IgM response, however, did not correlate with the response of any other antibody classes measured. Because the hemocyanin molecule contains carbohydrate structures,³ a T cell-independent response in the IgM class can be evoked by the carbohydrate part of the antigen. This could explain both the dissociation between the response in the IgM and in the other Ig classes and also the occurrence of IgM antibodies before immunization with HPH. The latter might belong to the so-called naturally occurring antibodies to common carbohydrate structures.⁷ Similar IgM antibodies to the primary antigens KLH^{13, 16, 17} and ØX 174¹⁸ have been found in the sera of unimmunized subjects. Earlier contact with the antigen seems unlikely, as levels of IgM antibody before immunization in our study do not correlate with the IgM antibody response after immunization.³

Immune responses induced by parenteral immunization might not be equivalent to the immunologic results of natural exposure to antigens in asthma. Recent studies report good correlations between the IgE or IgG (or IgG₄) antibody level and purified allergens after natural exposure in patients with asthma.^{2, 19-22} This suggests a high degree of linkage between the IgE and IgG antibody response in asthma not only after parenteral immunization but also in natural conditions.

In addition to the high level of HPH-specific IgE antibodies, there were increased levels of serum IgE in the patients. However, the high antigen/allergen-specific IgG antibody response did not result in an elevated level of total serum IgG. Differences in the catabolic mechanisms for IgG and IgE might partially explain this. IgG catabolism is related to the total plasma concentration²³ and is therefore increased when the plasma concentration rises. IgE catabolism, however, depends partially on receptor-mediated processes.²⁴ Therefore one might speculate that receptor

saturation limits the capacity of this mechanism to cope with an increased supply.²⁵ Moreover, the relative contribution of specific antibodies induced in patients with asthma after contact with an allergen/antigen to the total IgE serum pool greatly exceeds that for IgG.

In conclusion, this T cell-dependent antibody response to newly encountered antigen appears to be greater in people with even mild asthmatic symptoms. This observation is not restricted to the IgE antibody class but involves the IgG antibody response as well.

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