

Specific IgE and IgG Responses in Atopic versus Nonatopic Subjects

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It is becoming increasingly clear that the asthmatic reaction is the common end point of different processes. These processes all appear to be multi-step reactions. Several of these reaction steps are known (or expected) to be under genetic control. This meeting is concerned with one step in one of these pathways: the development of IgE antibodies to airborne allergens. The rationale for this focus is that this is likely to be an important (if not essential) step in most cases of asthma in the 4- to 40-yr age group. It is obviously not the only step, because most subjects who become so sensitized do not develop asthmatic symptoms on allergen exposure. Factors that contribute to the localization and severity of the subsequent reaction include the following: the extent of the activation of inflammatory helper T type 2 (Th2) cells and eosinophils, the migration into and retention inside the lung tissue of these activated cells, and the induction and repair of the tissue reaction.

TOTAL IgE

The analysis of genetic factors that are involved in asthma is complicated by the above-mentioned multiplicity of critical steps. Furthermore, there is an interaction between some of these processes. Activated Th2 cells are not only important as effector cells in the inflammatory reaction, but also for the class switch in B cells. The association between total IgE and asthma is largely independent of the association between allergen-specific IgE and asthma. The former is a reflection of the threshold for the production of relatively large amounts of Th2 cytokines (many of which are important both as inflammatory mediators and as B cell switch factors), the latter reflects a particular type of immune responsiveness. It is to be noted that the median level of total IgE is typically 200–400 kU/L in uncomplicated atopic disease. A total IgE of more than 1,000 kU/L suggests a combination of abnormalities. For the study of the genes involved in atopy it would be preferable to focus attention on subjects with total IgE below 800 IU/L. It could be argued that a high total IgE and multiple sensitization are consequences rather than causes of severe (i.e., Th2-driven?) asthma (1). This line of reasoning invites the (as yet unanswerable) question on how many asthmatics do *not* develop multiple sensitization despite sufficient exposure to multiple allergens.

THE NONATOPIC IgE RESPONSE

The definition of atopy is debatable, but for the current discussion it will be used to indicate a genetic predisposition to mount an IgE response to low levels of airborne antigens, typically pollen and mite allergens. According to this definition, not all IgE responses are atopic. Few would argue that IgE responses to

helminths or castor beans are atopic, but some other situations are less evident. The risk of developing IgE antibodies to rodent urinary proteins after occupational exposure is influenced by, but not strictly dependent on, an atopic background (2). The same holds for the risk of developing IgE antibodies to tetanus or diphtheria after routine prophylactic vaccination (3).

In nonatopic subjects IgE responses require a strong antigenic stimulus (tetanus vaccine, insect venom, occupational exposure to airborne allergens, helminths). These nonatopic IgE responses result in IgE antibody levels in the same range as in “classic” atopic IgE responses (1–100 ng/ml) or, in special cases, even higher, for example, in helminth infestation. In some cases allergic reactions are as severe as in atopic patients, but in many cases the lack of allergic symptomatology is striking. This lack of symptomatology is likely to be at least partially due to high levels of protective (“blocking”) IgG antibodies.

THE ATOPIC IMMUNE RESPONSE: NOT JUST IgE

If the concept of nonatopic IgE responses is accepted, the immunological differences between atopic and nonatopic IgE responses become of interest. In this respect it is relevant to look not only at the IgE antibodies in these two types of immune responses, but also at the other isotypes. The basic observation in this respect was made by Platts-Mills (4), who observed a close association between the presence of IgG antibodies to a purified grass pollen allergen (*Lol p 1*) and IgE to that allergen. The same was true of IgA antibodies. Similar results were obtained by Chapman and colleagues (5) using a purified mite allergen (*Der p 1*). The absence of IgG (and IgA) antibodies in nonallergic subjects is striking. This concordance between the presence of IgG and IgE antibodies is not found with other antigens such as those mentioned before (tetanus, insect venom, occupational allergens, helminths). In the latter situation many subjects will have IgG antibodies in the absence of demonstrable IgE antibodies.

In the author's view the presence of the “atopy gene” (or genes) is a facilitating rather than an enabling factor: An individual does not need this gene to make IgE antibodies, but is more likely to make IgE antibodies if it is present (i.e., this gene). The stronger the antigenic stimulus, the higher the chance of making IgE antibodies. In this stochastic view, the effect of the gene is a shift in the dose–response curve toward the left (i.e., an increased risk of developing reactivity at low levels of stimulation). One possible explanation of the current increase in the incidence of allergic sensitization is that the dose–response curve in the general population is shifting to the left, that is, the risk of making an IgE response at a low level of antigenic stimulation increases, particularly in the nonatopic group.

IgG TO AIRBORNE ALLERGENS IN NONATOPIC SUBJECTS

As mentioned before, IgG antibodies to purified pollen or mite allergens is closely associated with IgE antibodies. This is obviously not found for all allergens; even for airborne aller-

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gens the results are not uniform. One of the exceptions has already been mentioned: In situations with a high level of exposure (e.g., occupational exposure to rodent allergens) IgG antibodies are found in virtually all subjects and no association between IgG and IgE antibodies is evident. Divergent results have been found for allergens with low levels of exposure (less than 10 µg of dust per gram). Depending on the procedure used for the IgG antibody assay (radioimmunoassay [RIA] versus enzyme-linked immunosorbent assay [ELISA], purified allergen versus total extract) IgG responses in IgE-positive and IgE-negative subjects may be markedly different (particularly when using an RIA with purified allergens, i.e., selecting for high-affinity antibodies) or similar (particularly when using an ELISA, which might detect antibodies with lower affinity, or antibodies to trace contaminants in the allergen preparation). The discrepancies between the results of different laboratories have not been fully resolved, but in the author's view the presence of high-affinity IgG antibodies to purified allergens in IgE-positive subjects (almost) exclusively is a characteristic of the atopic immune response.

IgE VERSUS IgG₁ and IgG₄

The proliferative potential of B cells seems to differ depending on the isotype. A low proliferative potential most likely reflects a bias toward differentiation into nonproliferating plasma cells. Extrapolating the *in vitro* data of Brinkmann and Heusser (6), IgE-switched B cells are much more likely to differentiate into plasma cells and thus less likely to proliferate than IgG₄-switched B cells, whereas IgG₁-switched B cells are intermediate. This would explain why IgG₄ antibodies become prominent only late during chronic antigenic stimulation. In view of the data suggesting a sequential switch via IgG₄ to IgE (7), this would be compatible with a short window period in which the antigen-stimulated B cell might switch/differentiate from an IgG₄-stage B cell to an IgE-producing plasma cell. Beyond this point the B cell will either become a long-lived IgG₄ memory cell or an IgG₄-producing plasma cell. This would explain why conventional allergen-specific immunotherapy has so little effect on the level of allergen-specific IgE and such a marked effect on the level of allergen-specific IgG₄. As was shown already in the early 1980s by Holt and coworkers (8, 9), IgE production is maintained after X-irradiation (800 or 1,000 R) of BALB/c mice, which suggests that IgE production (at least in this rodent system) is largely due to long-lived plasma cells (for a review on the longevity of a subpopulation of plasma cells, see Reference 10).

A marked difference between IgG₁ and IgE (and, to a lesser extent, IgG₄) is the amount of "nonspecific" or "bystander" immunoglobulin production, as is evident from a comparison between the proportion of allergen-specific IgG₁ and IgE (relative to the total IgG₁ and the total IgE, respectively). This indicates that (in general) allergen is essential to induce B cells to switch to IgE, presumably because a cognate interaction between T cell and B cell is required to ensure an efficient focusing of T cell-derived cytokines onto the B cell. Only in exceptional situations (such as helminth infection) may IL-4/13 production be sufficient to achieve switching to IgE without cognate T-B interaction. This generates the paradox that IgE responses to allergens on the one hand are tightly regulated, but on the other hand are highly polyclonal (11).

ALLERGENS FROM THE POINT OF VIEW OF THE IMMUNE SYSTEM: DANGEROUS OR TRIVIAL?

As has been argued eloquently by Matzinger (12), the immune system is very much focused on a subset of potential antigens,

such as microbial antigens. Such antigens are recognized not only by the immune system, but also by other cells reacting to "danger signals" such as bacterial cell wall components. It has been argued by several authors that allergens are often enzymes (for a review see Reference 13) and that this activity might have a special effect on the immune system. The best known example is the group 1 mite allergen, which is known to be a cysteine protease. This has been reported to interact with the immune system in various ways, such as cleavage of CD23 and CD25 (for a review, see Reference 14). However, many allergens have no known enzymatic activity; some (e.g., lipocalins, such as the major urinary allergens) are pheromone-transporting proteins, unlikely to have dramatic intrinsic effects on immune cells.

An alternative hypothesis would be that the critical property of allergens is as follows: the absence of "danger" properties. The immune system does not care about such trivial substances and either does not react at all (ignorance) or reacts with a weak response according to the default (i.e., Th2) pattern. The stronger the stimulus (i.e., the more "dangerous" the antigen), the stronger the Th1 component of the response.

WORKING HYPOTHESIS: THE DIFFERENCE BETWEEN AN ATOPIC AND A NONATOPIC IgE RESPONSE

An atopic IgE response is a response to a low-grade ("trivial," as opposed to "dangerous" in the sense conveyed by Matzinger [12]) antigenic stimulus. If such a weak stimulus happens to induce any response at all, it defaults to Th2, with IgE responses in the same (low nanogram) range as IgG responses. In contrast, a normal IgE response requires a stronger antigenic stimulus, which will virtually always result in substantial quantities (ranges, 100–10,000 ng) of IgG antibodies and may result in IgE antibodies (low nanogram). Atopic subjects are exceptionally prone to mount an immune response to a trivial antigenic stimulus.

CONCLUSION

For the identification of polymorphic genetic factors that are involved in the processes that lead to allergic asthma it is important to keep in mind that not all IgE antibody reflects the activity of atopy genes. Sensitization of nonatopic subjects is clearly possible, but this requires higher and/or longer antigen exposure than sensitization of atopic subjects.

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DISCUSSION

Djukanovic: What do you think of the role of IgE in tetanus, is it just some sort of by-product with no part in the protective effect of the vaccine?

Aalberse: I think it is a nonprotective by-product. A normal immune response will include many isotypes, so why not IgE. The switch to IgE is not an easy one, as we discussed yesterday, so it occurs with a relatively low frequency. If you compare IgE to IgG in tetanus, the ratio is low, 1 in 1,000 or less.

Björkstén: I subscribe to what you are saying about the IgE response in relation to vaccination. We have gone through all available vaccines to pertussis that are on the market, including the component vaccines, as well as whole cell vaccines, with or without adjuvant. Certainly, there are very large IgE responses and they are fairly common. They are absolutely not related to atopy at all.

Aalberse: I don't agree there. There is a weak correlation.

Björkstén: They are not related to disease, I should say, but the levels tend to be slightly higher if you are an atopic individual. But what we consistently see, with tetanus, diphtheria, and pertussis, is that there is a relationship between side reactions measured by the size of the infiltration at the site of the injection. The pertussis data were obtained as part of those big placebo-controlled studies in Sweden. It involved both acellular and whole cell vaccines, one with an adjuvant and the other without adjuvant. In principle the patterns are similar, but more pronounced reactions were found with the acellular vaccine.

Aalberse: But alum does not make a difference?

Björkstén: No, it does not. You cannot blame the alum. Based on the animal experiments by Pauwels we assumed that alum would be an adjuvant for IgE, but it was not. The only thing that came out was that the acellular vaccines were stronger, particularly when you have the primary immunization with an acellular vaccine.

Kauffman: Do you agree that the observation that both IgG and IgE are generated against house dust mites can be used as an indicator that in asthmatics an abnormal (high) presentation of HDM antigen to the immune system occurs? This would support the hypothesis that epithelial behavior in asthmatics is abnormal/disturbed.

Aalberse: I think that the level of exposure to mite allergens is really very low. It is the lower limit of what you will find for immune responsiveness. Most people do not react at all. In my view the absence of IgE or IgG in the bulk of the population is

not tolerance, but just ignorance. I am not sure Dr. Holt will accept that. However, there has to be a certain level below which there is not any immune reaction at all. We may debate where this level is, but just above this threshold the IgE and IgG responses are at the same level. When you increase the amount of antigen, IgG will keep on rising, whereas IgE levels off.

Platts-Mills: it is quite clear that there is a relationship between total IgE and asthma. In several of our studies we see that grass pollen IgE correlates with hay fever but does not correlate with asthma. Mite or cat allergen correlates with asthma and total IgE also correlates with asthma. It is as if relevant long-term allergens boost total IgE more than seasonal allergens.

Aalberse: We do not find major differences between total IgE in relation to IgE to either grass pollen or mites. Total IgE is a bit higher when there is IgE to cats, which might support the idea that perennial exposure boosts total IgE, but in this case the median level goes from 300 to 600 IU/ml. Most of the studies are done with high-risk children who have both specific IgE and a strongly elevated total IgE, which makes it very difficult to separate the two. In the genetic study by Marsh and coworkers, the genetic effect was much more clear-cut when they removed all the cases with specific IgE from the statistical analysis before they looked at differences in total IgE. Specific IgE was a confounder; it blurred the genetic effect on total IgE. I think that the idea that total IgE is a marker of cytokine overproduction makes sense in a view of the data that we now have. Would you agree that if you concentrate on cases with total IgE below 500 you would have a much cleaner system to look at, whereas if you have only IgEs of over 1,000 IU/ml, you would have a very different population?

Platts-Mills: That is definitely true, but we have to remember that the half-life of IgE in plasma is extremely short, so minor variations in that half-life can produce quite major differences. You are dealing with a system that is not like IgG at all.

Jansen: Is allergic asthma due to inhaled allergens really a risk factor for HMW-related occupational asthma? In other words, is it the result of a defect in the regulation of the local immune response, or is it a result of the allergic asthma? In the latter case it would be a matter of type and amount of deposited antigen inducing the same sort of immune reactivity.

Aalberse: I am not sure whether the animal house workers were differently exposed. What I do know is about tetanus, where the dose is the same and still you find that people with an atopic background give a higher response on average than people without atopy. So in this case it is not a difference in antigen dose, but an immune regulation effect.

Aalberse: May I ask the audience a question. If you look at age-20 kids and you would have the option of switching-off IgE at an early age, say age below 1, what would be the impact for asthma at age 20? Would asthma at age 20 largely disappear or would the effect be trivial? My personal estimate is that 80% of asthma at age 20 would be gone, even though it may show up later.

Holgate: We can't answer your question yet, but we do know quite a bit about the adult side from the clinical trial with the anti-IgE antibody E25 in a very tight patient population by IgE levels and by skin tests, even though the asthma was variable. It showed that a third of the patients did really well, a third of them did so-so, and a third did nothing at all. And yet the investigators could not identify any phenotypic markers in these asthmatic patients that demonstrated that they were any different. That begs the question about the gradation of the processes. When any of these reagents becomes available we can begin to tackle these questions.

Aalberse: Yes, the real answer will come only then, but if you don't feel confident that it might work, then no one is going to

do it, because it is an aggressive treatment. Would we be willing to use an aggressive treatment?

Holgate: I would love to do it.

Holt: The reason why Novartis did not have more success could be because the effect of IgE in pushing the syndrome

alone may be over and done with after 5 to 10 years of assault on the airway mucosa. So, if that was the case, it is in essence a different disease process. The study you suggested is the only way to address that possibility.