

Evidence that negative feedback between antibody concentration and affinity regulates humoral response consolidation to a non-infectious antigen in infants

Duaine R. Jackola^{a,*}, Carol L. Liebeler^a, Ching-Yuang Lin^b, Yi-Kai Chiu^c,
Malcolm N. Blumenthal^a, Andreas Rosenberg^a

^a The Asthma and Allergy Center, University of Minnesota Medical School, Minneapolis, Mayo Mail Code 434,
420 Delaware St. S.E., Minneapolis, MN 55455, USA

^b Children's Hospital, Changua Christian Hospital and Institute of Medical Research,
Chang Jung Christian University, Taiwan

^c Institute of Microbiology and Immunology, National Yang-Ming University, Taiwan

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Abstract

The dynamics of human antigen-specific immunoglobulin (Ig) responses in early life are not well characterized. We have previously observed an inverse relationship between allergen-specific Ig concentration and allergen-Ig-binding affinity in allergen-sensitive atopic adults, suggesting a possible feedback relationship between these variables. We prospectively studied children (6 months to 6 years) with and without atopic sensitization to the Der p 1 major allergen. Experimental results showed the following trends. (1) In both study groups, there was little change with age in average Der p 1-specific Ig (IgG1 or IgE) concentrations or allergen-Ig-binding affinities, and concentrations and affinities were independent. (2) Among individuals, however, there was a negative correlation between Ig concentration changes and affinity changes with age. (3) The rate of increase with age of the non-atopic Der p 1-IgG1 total binding capacity (Ig concentration $\times$ Ig affinity) paralleled that for the atopic Der p 1-IgE total binding capacity, and there was a comparable 'consolidation' of responses with age reflected by a narrowing of the variance of total binding capacity values. Except for the Ig classes involved, development of a humoral response to a non-infectious allergen is similarly regulated in atopic and non-atopic children, with Ig total binding capacity as the key regulatory variable. These results also suggest that there is a time-dependent feedback relationship between Ig concentrations and affinities that establishes an optimal Ig total binding capacity for a given environmental 'antigen load'. A theoretical model is proposed to account for this relationship.

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1. Introduction

Prolonged exposure to antigens engenders fundamental changes in T-cell-dependent humoral responses. Based upon evidence derived from animal models and in vitro cell culture (Steward and Steensgaard, 1983; Cerutti et al., 1998), a great deal has been learned about the key mechanistic fea-

tures. These involve structural and functional changes of immunoglobulins via the complementary activities of somatic hypermutation (SH), leading to antibody 'affinity maturation', and isotype class switch recombination (CSR) that promotes specific functional changes upon secondary antigen exposure(s) (Wabl and Steinberg, 1996; Kouskoff et al., 1998; Stavnezer, 2000; McHeyzer-Williams et al., 2001). Ultimately, the interplay of SH and CSR progressively leads to the most energetically favorable repertoire for a particular challenge (Berek and Ziegner, 1993; Hodgkin, 1997),

* Corresponding author. Tel.: +1 612 626 4624; fax: +1 612 624 9188.
E-mail address: jacko001@umn.edu (D.R. Jackola).

represented by a pool of terminally differentiated, antigen-specific memory B and plasma cells (McHeyzer-Williams and Ahmed, 1999).

However, little is known regarding the dynamic outcomes of these processes in humans during the course of natural exposure to environmental allergens, a major source of persistent antigen exposure, particularly during the earliest post-natal years when antigen encounters first occur. Maturation of the immune system during the earliest years of life is critical for establishing normal cellular and humoral responses; else pathologies may ensue upon exposure to otherwise innocuous environmental challenges.

Given a certain ‘antigen load’, the most suitable physiological effectiveness (ability to remove antigen from the system) may be engendered either by increasing the production of specific antibodies (concentration) or enhancing the antigen–antibody-binding properties (affinity). Further, if the ‘antigen load’ is relatively constant, then there should be some optimal combination of concentration and affinity resulting in the most energetically efficient use of resources for the given challenge (a total antigen binding capacity).

Although in vitro evidence has shown that SH precedes CSR (Liu et al., 1996), suggesting that the two are mechanistically independent of one another, they are not mutually exclusive processes for dealing with an antigen challenge, which proposes that there is an interplay between the two, possibly via a feedback mechanism. The most readily measured product of the humoral response, antibody concentration, may not be a suitable independent variable, as we have observed among adults that there is an apparent inverse relationship between concentration and the antibody-binding affinity for allergens (Pierson-Mullany et al., 2002). However, this observation suggests that there may be a negative feedback relationship involved.

Among some people, common environmental allergens produce a pathologic response involving the production of allergen-specific IgE (Blumenthal and Björkstén, 1997). Aside from the pathologic consequences, however, allergens are ideal models for the humoral responses that develop due to prolonged exposure to non-infectious antigens. We have shown among adults, either atopic or non-atopic, that there is a dynamic interplay between a response ‘potential’, reflected by the type and amount of specific immunoglobulin produced, and the ‘driving force’ of the antibody, reflected by its binding affinity for the antigen/allergen that induced its production (Jackola et al., 2002). There was an inverse relationship between optimal specific immunoglobulin concentration and its binding affinity, suggesting, again, a feedback regulation leading to a most efficient total binding capacity. However, the age-dependent progress of immunoglobulin changes in the earliest years of life leading to these outcomes has not been established.

House dust mite allergens, like Der p (*Dermatophagoides pteronyssinus*), are common, non-infectious allergens that provide an essentially constant environmental ‘antigen load’. We prospectively followed two groups of children from Tai-

wan, with and without Der p atopic sensitivities, for development of antibodies specific to the Der p 1 major allergen. We report here the changes that occurred with age in the Der p 1-specific immunoglobulins that these groups of children produced. We focused upon the dynamics of the changes in specific immunoglobulin concentrations and binding affinities and, in particular, the possibility of feedback interactions between these variables in the presence of the presumed constant antigen challenge.

2. Methods

2.1. Study population and screens for atopy

As part of an ongoing prospective study of the etiology of house dust mite (Der p; *D. pteronyssinus*)-associated atopic disorders in Taiwan, two groups of unrelated children were randomly selected, 15 children per group. Children’s parents provided written informed consent according to the guidelines of the Institutional Review Board, Changua Christian Hospital, Changua, Taiwan. Atopic children had characteristic symptoms of atopic dermatitis and/or bronchial asthma and had positive serum screens for Der p-IgE production, using purified Der p extracts with a Pharmacia-CAP[®] analyzer (lowest detection limit = 0.35 IU/ml; 1 IU = 2.42 ng). Non-atopic children had no symptomatic evidence of Der p-associated or other allergen sensitivity and serum screens were negative for Der p-IgE. In addition to cord blood samples, each of the children was screened for Der p sensitivity at ages: (1) 6 months to 2 years, (2) 2–4 years and (3) 4–6 years.

2.2. Serum immunoglobulin assays

Whole blood was obtained by sterile venipuncture. Serum was separated and stored frozen (–80 °C) until assayed. Quantitative determinations for allergen-specific immunoglobulin concentrations were made, and allergen-immunoglobulin-binding affinity distributions were done using an assay of our device (Pierson et al., 1998).

2.2.1. Der p 1-specific immunoglobulin assays

Der p 1 is a major allergen that was purified by methods previously described (Pierson et al., 1998). The purified Der p 1 was used in a ‘reverse sandwich’ ELISA assay for tests of Der p 1-specific IgE and IgG1 as described (Jackola et al., 2002).

2.2.2. Binding affinity distribution functions

Our basic experimental method provides information regarding allergen–antibody (Ag–Ab)-binding reactions in vitro (Pierson et al., 1998). Briefly, flat-bottomed microtest plates (Costar #3912, Corning, Inc., Corning, NY) are coated with a mouse anti-human, anti-IgE (clone GE-1, Sigma, St. Louis, MO) or anti-IgG1 (clone 8C/6-39, Sigma) monoclonal antibody (mAb). To this is added a serum sample known to

contain allergen-specific IgE or IgG, which is “captured” by the bound mAb. Biotinylated, purified allergen (BT-Der p 1) is added to bind only to the specific IgE or IgG1 of interest, and detection is achieved by the addition of streptavidin-conjugated alkaline phosphatase (Sigma) and a chemiluminescent substrate to generate a light signal.

Using a range of purified Ag (BT-Der p 1) concentrations, a binding isotherm is generated: r versus $\log_{10}[\text{Ag}]$, where r is the fraction of antibody sites bound at a given $[\text{Ag}]$ concentration. To eliminate “background noise” from the positive test signal, due possibly to non-specific binding of the Ag, parallel determinations were made for each Ag concentration using conditions that did not include Ag-specific IgE or IgG1. The background was subtracted from the test signal prior to determining the fraction specifically bound, r .

Because the purified allergens may have multiple epitopes, one or more specific Ag–Ab reactions may contribute to the resultant binding curve. However, the number (n) of reactions that contributed is not known or assumed a priori. We have developed a method to analyze this problem (Pierson et al., 1998) using an approach first suggested by Hunston (1975) and extended by Thakur et al. (1980).

The information from the binding isotherm is transformed to derive an affinity distribution function. These functions identify the number of reactions involved between an allergen-specific immunoglobulin and the allergen (n), the equilibrium-binding affinity constant for each reaction (K_A) and the relative proportion of each reaction to the sum of all reactions involved ($p[K]$). The data were analyzed using Mathematica® software (Wolfram Research, Inc., Champaign, IL).

Among dust mite sensitized adults, in all cases we have found two specific reactions between Der p1 and IgE antibodies and three specific reactions in about 2/3 of the cases (Pierson et al., 1998; Pierson-Mullany et al., 2000, 2002). Each of these reactions is directed towards a specific epitope of the major Der p 1 allergen (Pierson-Mullany et al., 2000). Representative affinity distributions may be found in Pierson et al. (1998) and Jackola et al. (2002).

2.3. Definitions

2.3.1. Average binding affinity

Two or more specific reactions may occur between allergens and allergen-specific antibodies, and each is characterized by a binding constant, K , and a proportionate response parameter, p , indicating a particular reaction's contribution to the total of all specific reactions involved. The average of all reactions is the mean binding affinity defined by: mean affinity = $\sum_{i=1}^n p_i K_i$, where n , p and K are defined above.

2.3.2. Total binding capacity

The binding affinity describes a reaction's ‘driving force’. The concentration of antibodies with these characteristics provides a particular response ‘potential’. The mathematical product of these two contributions is the antibody's total

binding capacity defined by: capacity = $[\text{IgX}] \times \sum_{i=1}^n p_i K_i$, where $[\text{IgX}]$ is the concentration of the particular antibody of interest.

2.3.3. Units

Binding affinity = L/M. Antibody concentration = M/L. Total binding capacity (concentration $\times$ affinity) is dimensionless.

2.4. A general equation for dynamic antibody capacity changes

A differential equation for the age-dependent dynamics of a variable, $Y(A)$, that reaches some limiting (asymptotic) value is given by: $dY/dA = \alpha - \beta Y$, where α and β are constants. The constant α reflects a general input value of the variable Y , and the constant β reflects the rate of change in the variable Y . A general solution is: $Y(A) = C e^{-\beta A} + (\alpha/\beta)$, where C is an arbitrary constant of integration. If $Y(0) = 0$, a particular solution is given by the following nonlinear equation:

$$Y(A) = \left(\frac{\alpha}{\beta}\right) (1 - e^{-\beta A}) \quad (1^*)$$

As $A \rightarrow \infty$, the asymptotic limit is given by (α/β) . It is assumed that all values $Y(A) \geq 0$. The scatter plots of Fig. 2, to be described in Section 3, have $\log[\text{binding capacity}]$ as the variable $Y(A)$, most of which values are <0 . We arbitrarily added a constant of 4.0 to all measured values so that all $Y(A) \geq 0$. These adjusted data were fit to Eq. (1*) using weighted least-squares and a nonlinear regression program that employed a Marquardt minimization algorithm (Bevington and Robinson, 2003). From these results, the estimated asymptotic values for $\log[\text{binding capacity}]$ were determined by: $(\alpha/\beta) - 4.0$.

3. Results

3.1. Within-study groups, Der p 1-specific antibody concentrations and affinities are independent variables

Table 1, part A, lists the results for Der p 1–IgE and IgG1 concentrations for the two groups of children at each of the ages when serum samples were obtained. Only the atopic children had evidence of clinical sensitization to Der p allergens, both by symptoms and serum clinical screens for Der p allergen-specific IgE. The non-atopic children were negative for these screens. Mean Der p 1–IgE concentrations increased with age among the atopic children, while there was no apparent increase in mean concentrations for Der p 1–IgG1 in either group.

Table 1, part B, lists the mean $\log(\text{binding affinity})$ values (defined in Section 2.3.1) for Der p 1–IgE (atopic) and Der p 1–IgG1 (atopic and non-atopic). As we have previously observed among adults (Pierson-Mullany et al., 2000), each

Table 1
Serum Der p 1 allergen screen results

(A) Allergen-specific antibody concentrations vs. age			
Test	Age (years)	Atopics	Non-atopics
Der p 1-IgE	1/2–2	4.1 (1.36) ^a	n.d. ^b
	2–4	12.7 (1.49)	n.d.
	4–6	16.5 (1.30)	n.d.
Der p 1-IgG1	1/2–2	17.4 (5.42)	13.9 (4.66)
	2–4	6.6 (1.99)	13.6 (7.06)
	4–6	8.5 (1.59)	19.6 (5.12)
(B) log[mean binding affinity] vs. age			
Der p 1-IgE	1/2–2	8.234 (0.123) ^c	n.d. ^d
	2–4	8.195 (0.129)	n.d.
	4–6	8.266 (0.140)	n.d.
Der p 1-IgG1	1/2–2	7.776 (0.197)	6.992 (0.524)
	2–4	8.011 (0.228)	7.632 (0.070)
	4–6	8.024 (0.187)	7.945 (0.129)

^a Mean value (S.E.M.); units = ng/ml.

^b n.d. = not detected.

^c Mean (S.E.M.); units = L/M (see Section 2.3.1).

^d n.d. = not detected.

child produced three epitope-specific antibodies (IgE and/or IgG1) for the Der p 1 major allergen. However, there were no apparent changes with age for mean log binding affinities in the study groups. Remarkably, the values listed in Table 1, part B, are comparable to values we have previously determined among both atopic and non-atopic adults (Jackola et al., 2002). Thus, based upon allergen-specific immunoglobulin (Ig) concentrations and average properties of Ig–allergen binding, children are able to mount a vigorous humoral response to common environmental antigens early in life.

To determine if there were relationships between the concentration and mean affinity values, we compared the two by simple linear regression (SLR):

Non-atopic :

$$\log[\text{mean affinity}] = 7.096 - 0.060 \times \log[\text{Der p 1-IgG1}],$$

$$F(1, 43) = 0.70, \quad P = 0.41$$

Atopic :

$$\log[\text{mean affinity}] = 9.729 + 0.187 \times \log[\text{Der p 1-IgG1}],$$

$$F(1, 43) = 1.94, \quad P = 0.17$$

$$\log[\text{mean affinity}] = 8.779 + 0.071 \times \log[\text{Der p 1-IgE}],$$

$$F(1, 43) = 0.56, \quad P = 0.46$$

In all three cases, the regression coefficients were not significantly different from zero. Although not shown, there were also no significant correlations found between these variables using the values at each of the three separate sample ages. These results suggest that antibody concentration and binding affinity are independent variables within the study groups.

3.2. Inverse relationship between Der p 1-specific antibody concentration changes and affinity changes with age among individuals

We have previously observed an apparent inverse relationship between allergen-specific antibody concentrations and binding affinities among adults 18 years and older (Pierson-Mullany et al., 2002). As shown above, we could find no apparent relationship for these variables within the study groups of infants. To determine if there might be any age-dependent developmental changes, we followed the time course of antibody concentrations and affinities for each child individually to determine if there were any apparent trends.

For each child, we determined the age-dependent changes, if any, for Der p 1-specific antibody concentrations and mean binding affinities using SLR for log-transformed variables. We then compared the “slopes” for concentration and mean affinity within each study group using simple correlation analysis. These results are shown in Fig. 1.

For Der p 1-IgG1 from non-atopic infants (Fig. 1, Panel A) and Der p 1-IgE from atopic infants (Fig. 1, Panel C), there were significant inverse relationships between age-dependent changes of concentration and mean binding affinities. These results suggest that, although the variables of concentration and affinity may be independently regulated, there may be an operational feedback on an individual basis during continuous exposure to environmental antigens. Interestingly, no apparent trend was found for Der p 1-IgG1 from atopic infants (Fig. 1, Panel B).

3.3. Age-dependent changes of Der p 1-specific antibody total binding capacities

As we have previously shown (Pierson-Mullany et al., 2002; Jackola et al., 2002), evaluating the total humoral response involves a dynamic interplay between the response ‘potential’ (Ig concentration) and the response’s ‘driving force’ (Ig–antigen binding affinity/avidity). The interaction between Ig’s and antigens from the environment is a function of both these variables: the total binding capacity (defined in Section 2.3.2).

Fig. 2 shows the dynamic changes with age for the total Der p 1 binding capacities for the two groups of children. Statistical comparisons between the groups are given in Table 2. At the earliest age (6 months to 2 years), the Der p 1-IgG1 capacities are higher among the atopic children compared to non-atopic values.

However, the Der p1-IgG1 capacity did not change with age for atopics, as assessed by SLR: $\log[\text{Der p 1-IgG1 CAP}] = -1.66 + 0.04 \times \text{age}$; $F(1,43) = 0.18$; $P = 0.68$. In contrast, there was a significant increase in Der p 1-IgG1 binding capacity with age for the non-atopic children. Additionally, the Der p 1-IgG1 capacity values approached an apparent asymptotic limit during the later years. The solid line in Fig. 2A is the best-fit result to the data for a simple nonlinear model accounting for an asymptotic limit (Eq. (1*)) defined

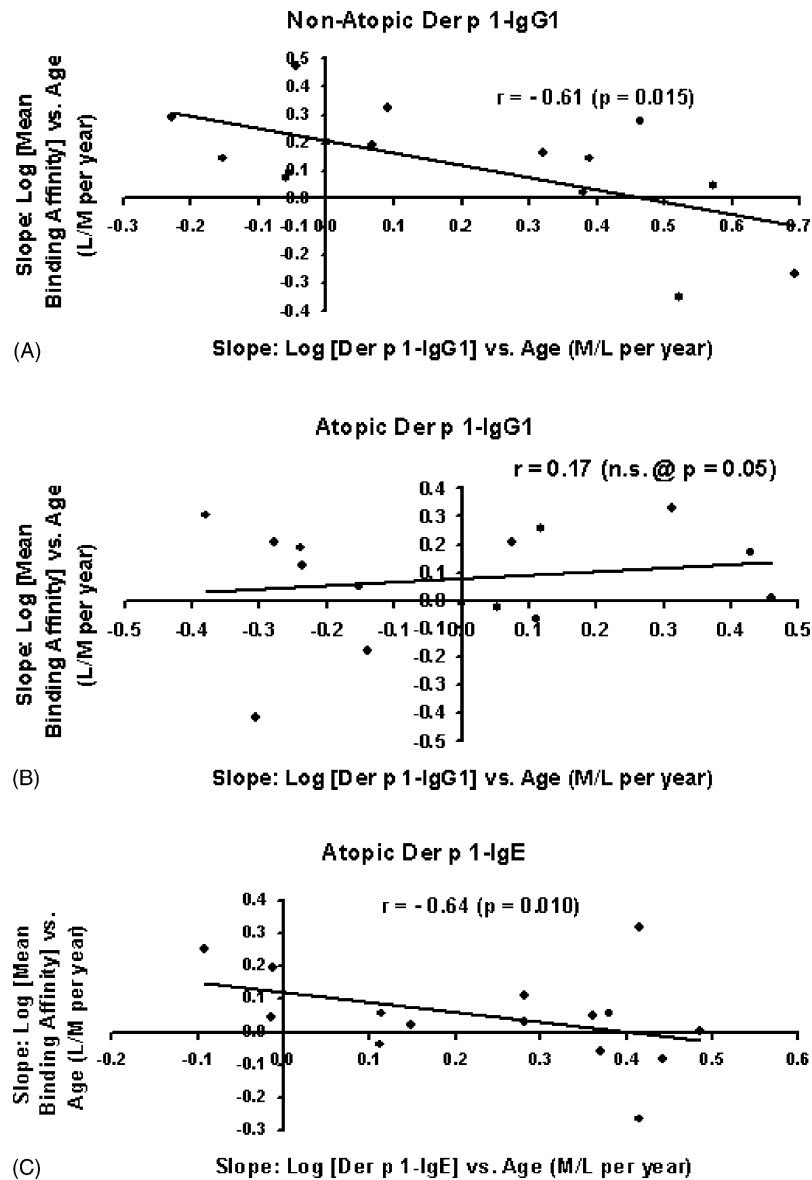


Fig. 1. Mean binding affinity changes vs. antibody concentration changes with age. For each child, we calculated the slope of the log[mean binding affinity] (Section 2.3.1) vs. age and the slope of the log[antibody concentration] vs. age. The slopes for mean affinity changes (ordinate) are compared with the slopes for the concentration changes (abscissa). The solid lines are the results of correlation analyses for these variables.

Table 2
Binding capacity variance stabilization with age

Age (years)	Atopic IgE-CAP	Atopic IgG1-CAP	Non-atopic IgG1-CAP
1/2–2			
Mean ^a	–2.666	–1.447	–2.208
Variance	0.566	1.636	2.792
2–4			
Mean	–1.801	–1.760	–2.063
Variance	0.352	1.830	1.641
4–6			
Mean	–1.571	–1.319	–1.173
Variance	0.080	0.643	0.377

^a log[binding capacity]; dimensionless units (see Section 2.3.2).

in Section 2.4) using weighted least-squares (Bevington and Robinson, 2003).

Fig. 2B compares the Der p 1-IgG1 and Der p 1-IgE binding values for the atopic children. The Der p 1-IgE capacity values also showed a significant increase with age, and approached an apparent asymptotic limit. This “rate” of increase was comparable to the Der p 1-IgG1 capacities from non-atopic children.

Fig. 2C compares the best-fit results for the Der p 1-IgG1 capacity values for non-atopic children and the Der p 1-IgE values for atopic children. We divided the age scale for the results in Fig. 2C into 24 equally spaced intervals, and compared the two best-fit curves at each of these intervals by χ^2 analysis: $\chi^2(23) = 0.89$; no significant difference at $P < 0.001$.

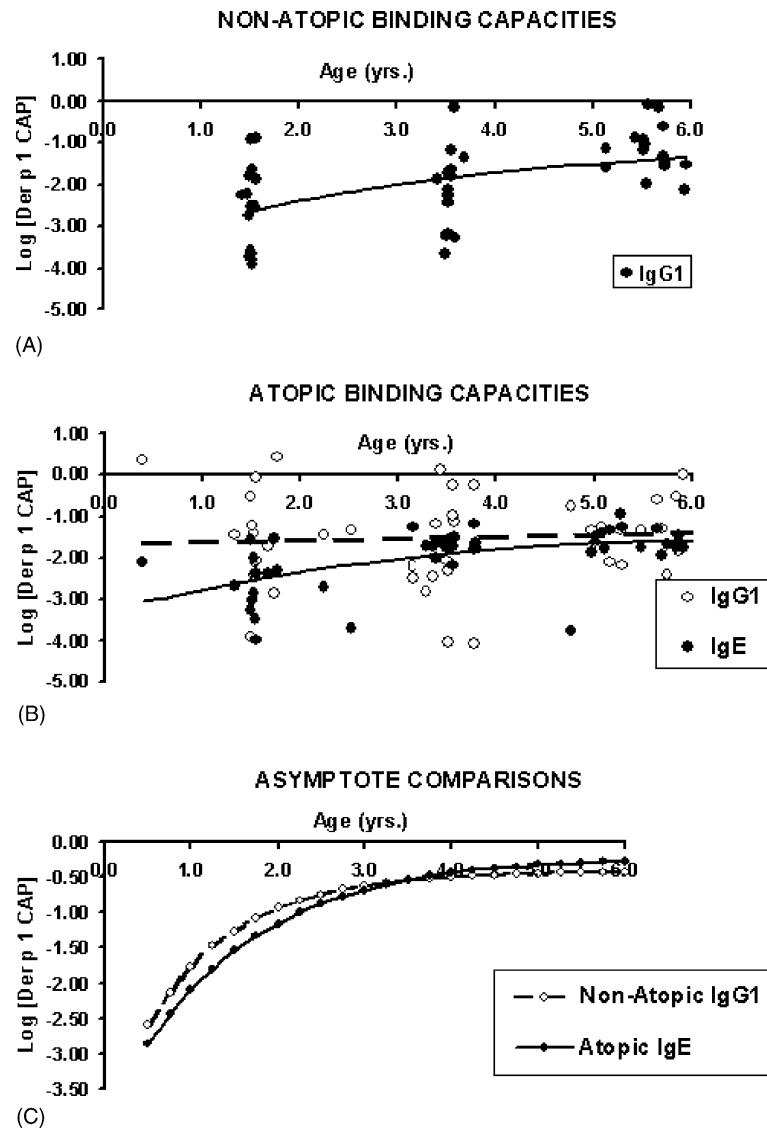


Fig. 2. Total binding capacity changes with age. Serum total binding capacities were determined at three different ages for 15 non-atopic and 15 atopic children (Section 2.3.2). (A) Der p 1-IgG1 capacities for non-atopic children. Solid line is best-fit result for a nonlinear model with an asymptotic limit (Section 2.4). (B) Der p 1-IgG1 and Der p 1-IgE capacities for atopic children. Broken line is best-fit SLR result for Der p 1-IgG1 vs. age. Solid line is best-fit curve of Der p 1-IgE results using a nonlinear model. (C) Comparison of nonlinear curve-fitting results for non-atopic Der p 1-IgG1 and atopic Der p 1-IgE capacity values. As described in Section 2.4, the asymptotic limit is given by the ratio of two constants (α/β), after data adjustment for the fitting algorithm: asymptote = $(\alpha/\beta) - 4.0$. Results for non-atopic Der p 1-IgG1: $\alpha = 3.491 \pm 0.367$; $\beta = 0.976 \pm 0.119$; $R^2 = 0.36$; asymptote = $(3.491/0.976) - 4.0 = -0.424$. Results for atopic Der p 1-IgE: $\alpha = 2.643 \pm 0.260$; $\beta = 0.697 \pm 0.086$; $R^2 = 0.47$; asymptote = $(2.643/0.697) - 4.0 = -0.208$.

The best-fit results are virtually identical suggesting similar, optimal humoral responses to common environmental allergen exposures.

As suggested by the results in Table 1, antibody concentration or antibody affinity taken alone appear to reflect an irregular behavior. Additionally, as shown above, these variables are statistically uncorrelated within the study groups, suggesting that they are independent of one another. However, for children individually, there is an inverse relationship between age-dependent changes of concentration and affinity (Fig. 1). Further, the combination of the two (total binding capacity) reflects a well-regulated progression toward an optimal, most efficient antibody response for the given antigen

challenge, regardless of the isotype involved. In this context, total binding capacity appears to be the key regulatory variable.

In Fig. 2, the variances of the distributions of values decline with age for both the log Der p 1-IgG1 and IgE values. From a basic evaluation of equilibrium binding between a protein (P) and its ligand (L), the association constant (K_A) is defined as: $K_A = [PL]/[P][L]$. The total ligand concentration in a reaction mix is: $L_{Total} = [PL] + [L]$. The fractional saturation of bound ligand is defined by

$$\gamma \equiv \frac{[PL]}{L_{Total}} = \frac{[PL]}{[PL] + [L]} \quad (1)$$

Algebraic rearrangement gives the following relation:

$$K_A[P] = \frac{\gamma}{1 - \gamma} = \frac{[PL]}{[L]} \quad (2)$$

where γ is the fractional saturation is always <1 since under equilibrium conditions $[PL]$ never equals L_{Total} . Defining the total binding capacity (CAP) by this relation

$$CAP \equiv K_A[P] = \frac{\gamma}{1 - \gamma}, \quad 0 < CAP < \infty \quad (3)$$

Although all values in this range are possible in theory, the evolution of a particular humoral immune response is impelled towards the most energetically efficient use of available resources for a given environmental challenge (Berek and Ziegner, 1993; Hodgkin, 1997). Thus, it seems unlikely that a humoral response would develop that would encompass all possible values for total binding capacity.

Table 2 lists the means and variances for the $\log[CAP]$ values. Clearly, there is a decrease with age in the listed statistical variances. To determine if this was due to some common physiological mechanism, we analyzed the ‘deviation’ from the mean value for each child at each age when a serum sample was obtained.

At each age, the mean $\log[CAP]$ value was subtracted from each child’s $\log[CAP]$ value to give a “deviation score”. A child’s value above the mean would translate to a (+) deviation score, while a value below the mean would translate to a (–) deviation score. Then, for each child, we determined the “slope” of these deviation scores with age by SLR.

Fig. 3 shows the scatter plots of these slopes compared with the initial deviation score (sample at 6 months to 2 years) for each of the children. Clearly, for each child, an initial (+) deviation score resulted in a downward trend in deviation scores with age {(–) slope}, while an initial (–) deviation score resulted in an upward trend of deviation scores with age {(+) slope}. For each child, the combined contributions of Ig production and binding affinity impelled the total binding capacity to more closely approximate the mean population value.

4. A feedback model for post-germinal center humoral response consolidation

Common indoor allergens like house dust mites provide a continuous source of low-level, non-infectious antigenic challenge. If we assume that the concentration of this challenge remains essentially constant, then it is also reasonable to assume that the immune response(s) to this challenge will eventually achieve some optimal level. A qualitative model for the changes with age in antigen-specific immunoglobulin responses must take into account three observations presented above.

First, within the study groups of infants (atopic and non-atopic), antibody concentrations and binding affinities

are statistically uncorrelated, suggesting that they are independent variables. Second, on a per child basis there are significant negative correlations of age-dependent changes between concentrations and their corresponding binding affinities (Fig. 1) suggesting that there is an operational feedback relationship between two independently regulated variables. Third, in terms of total binding capacity, there are similar age-dependent trajectories for the changes of atopic Der p 1–IgE and non-atopic Der p 1–IgG1 (Fig. 2C) and the statistical variances of the capacity values decline as a function of age (Table 2), suggesting that there is an optimal antigen-specific repertoire for a given antigen challenge.

The key response variable is the total binding capacity with respect to its efficiency for clearing the antigen challenge from the system. We previously defined γ as the fraction of bound ligand (antigen/allergen). From Eq. (3) above, the ratio of bound ligand (γ) to unbound or free ligand ($1 - \gamma$) describes the total binding capacity. Fig. 4 shows a schematic of a simple “feedback loop” (Hobbie, 1997) for a model that incorporates these assumptions and observations.

After development of B-cells specific for a particular antigen challenge within the germinal centers, terminally differentiated memory B or plasma cells are available for subsequent antigen challenges (Liu et al., 1996; McHeyzer-Williams and Ahmed, 1999; McHeyzer-Williams et al., 2001). The major component of the B-cell receptor (BCR) complex is antigen-specific, membrane-bound Ig (mIg) (Alès-Martinez et al., 1991). We assume that there are distributions of cells that vary with regard to their affinity for antigen and their ability to produce antigen-specific antibodies. The question becomes what is the best combination of antibody concentration and/or antibody affinity and how are these regulated to achieve the optimal binding efficiency (capacity) for a given challenge?

Under equilibrium conditions it is known that

$$K = \frac{[Ag-Ig]}{[Ag][Ig]} = \frac{CAP}{[Ig]} \quad (4)$$

where $[Ag-Ig]$ is an antigen–immunoglobulin complex and $[Ag]$ is the free antigen concentration. Assuming a constant free antigen concentration, $[Ag]$, and an optimal binding efficiency, CAP, there is an inverse relationship between the binding affinity constant, K , and the antigen-specific antibody concentration $[Ig]$. This is depicted in the upper panel of Fig. 4 using an arbitrary value for CAP.

Antigen bound to mIg on B-cells will induce the production of some amount of soluble immunoglobulin, sIg (Alès-Martinez et al., 1991). A simple relationship describing this, assuming some maximal level of production is

$$[sIg] = P_{\max} \frac{f[Ag]K}{1 + f[Ag]K} \quad (5)$$

where $P_{\max}$ is a simple proportionality constant for maximal production, f the fraction of free antigen bound to mIg, $[Ag]$ the free antigen concentration and K the affinity of sIg for the

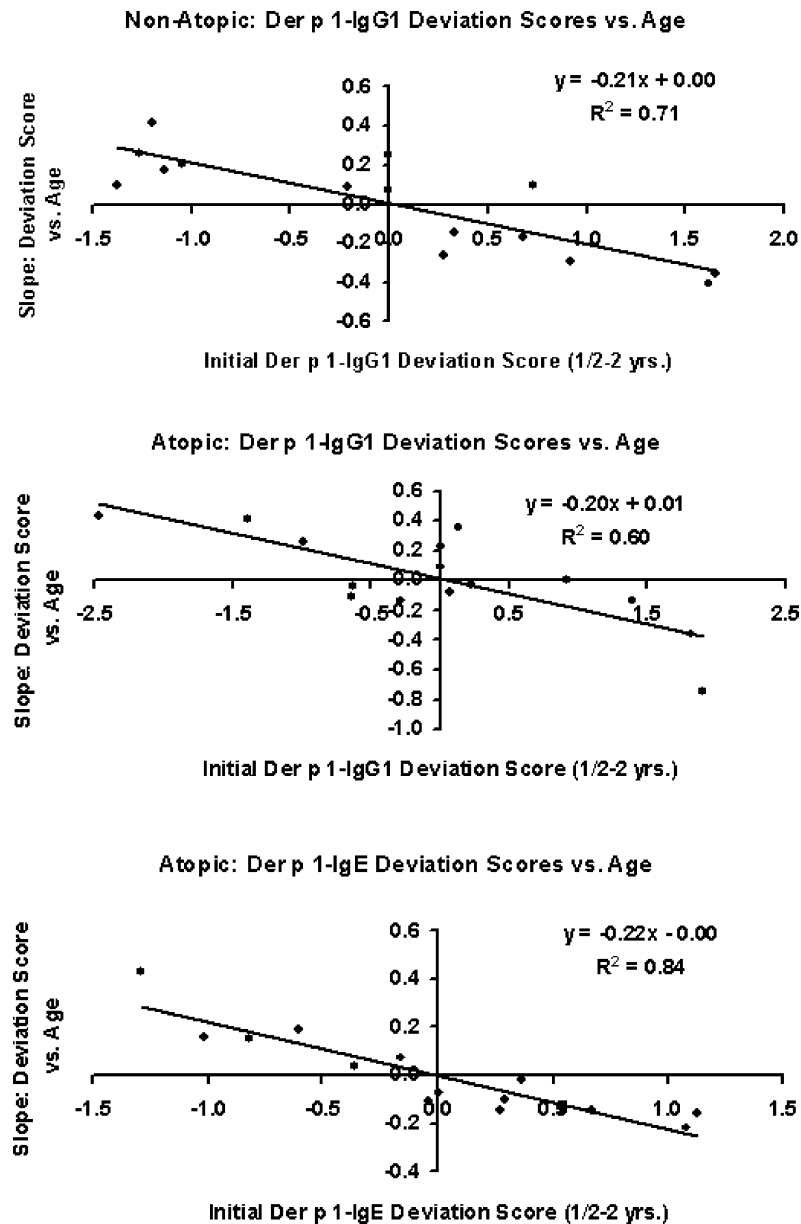


Fig. 3. Total binding capacity 'deviation scores' with age. At each age when a serum sample was obtained, the mean log[CAPACITY] value was subtracted from each child's log[CAPACITY] value to give a 'deviation score'. The 'slopes' of the deviation scores vs. age were determined for each child by SLR. These 'slopes' were compared with the initial sample deviation score (sample at 6 months to 2 years). The solid lines give the trends of the slopes vs. the initial deviation score as determined by SLR.

antigen. Assuming that everything but the affinity variable remains constant, this simplifies to

$$[sIg] = \frac{\delta K}{1 + \delta K} \quad (6)$$

This relationship is shown in the lower panel of Fig. 4.

Eqs. (4) and (6) are implicitly "linked" via the presumed constant free antigen concentration, [Ag]. For the system as a whole it is assumed that there is also some optimal binding efficiency (CAP) involving the antibody concentration and its affinity for the antigen that will remove the antigen from the system (small box at bottom of Fig. 4). This opti-

mal point is found when Eqs. (4) and (6) are solved simultaneously. A schematic of this "operating point" is shown in Fig. 5 where Eqs. (4) and (6) are plotted on the same set of axes.

When antigen first enters the system, it will bind to cells having variable affinities for antigen. This will lead to the production of some amount of sIg, depending upon this distribution of affinities. This will define some binding capacity that may or may not be at the "operating point" for the system. Following this initial round, either more sIg is produced or the cells with variable affinities are differentially selected or both simultaneously during successive "rounds" in the

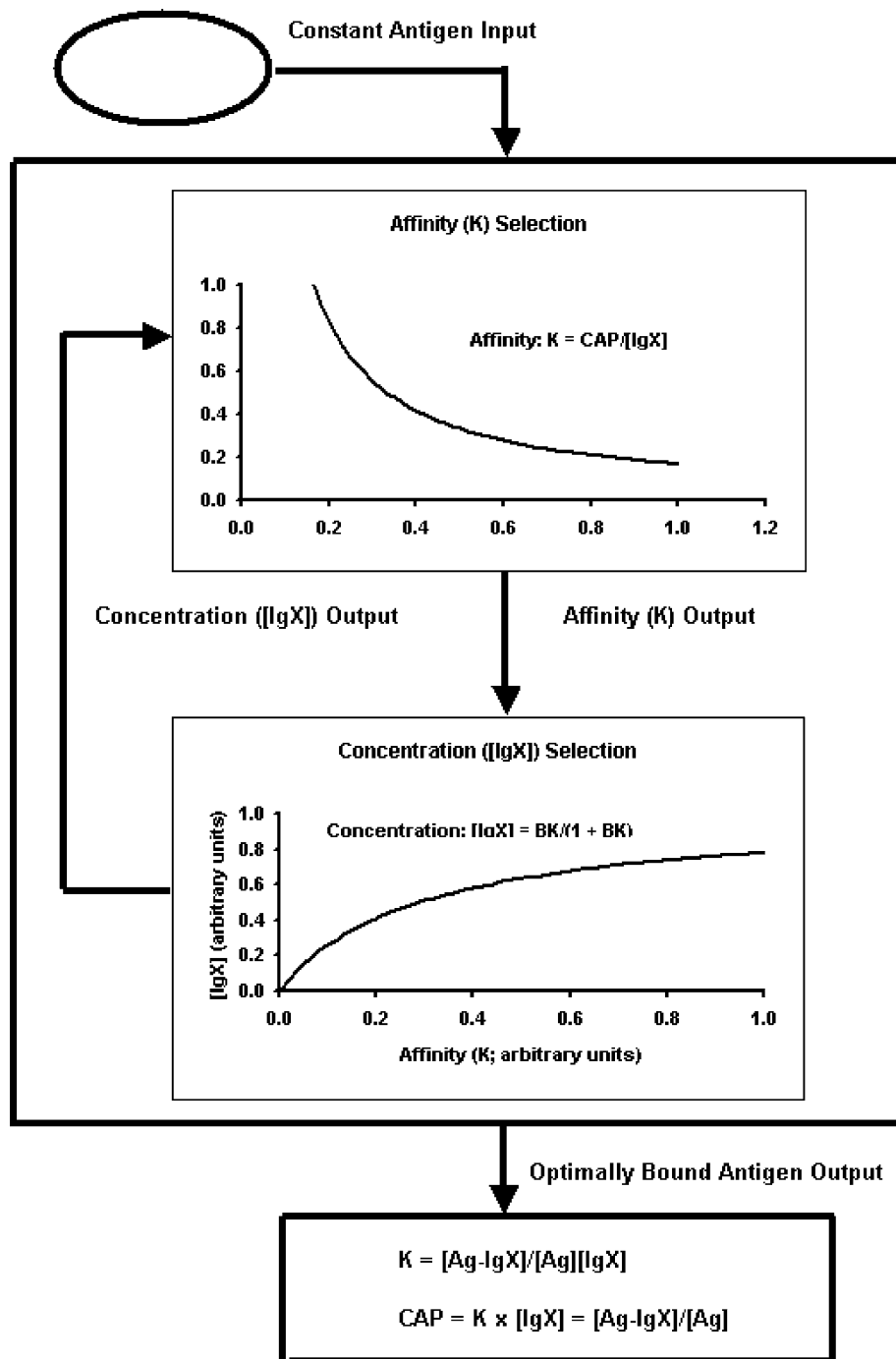


Fig. 4. Feedback model for antibody selection. Assuming a constant level of antigen input (oval at top), there will be some optimal antibody concentration and its associated binding affinity for the antigen challenge (lower panel). Initially there will some distribution of memory B or plasma cells that vary in their ability to produce antibodies with different antigen binding affinities and/or antibody concentrations. As discussed in Section 3, the plots in the middle panel depict a feedback relationship for selection of these cells that will ultimately achieve the optimal binding efficiency (total binding capacity).

feedback loop. To determine if there is zero, negative or positive feedback, we must determine the “gains” in each part of the feedback system (Hobbie, 1997).

Gain is defined as the rate of output (dependent variable) relative to the rate of input (independent variable) in the different parts of the system. For the upper panel of Fig. 4 (labeled “affinity (K) selection”), the first gain is defined by the

relationship in Eq. (4):

$$G1 = \frac{dK}{d[\text{Ig}]} = -\frac{\text{CAP}}{[\text{Ig}]^2} \quad (7)$$

For the lower panel in Fig. 4 (labeled “concentration selection”), the second gain is defined by the relationship in

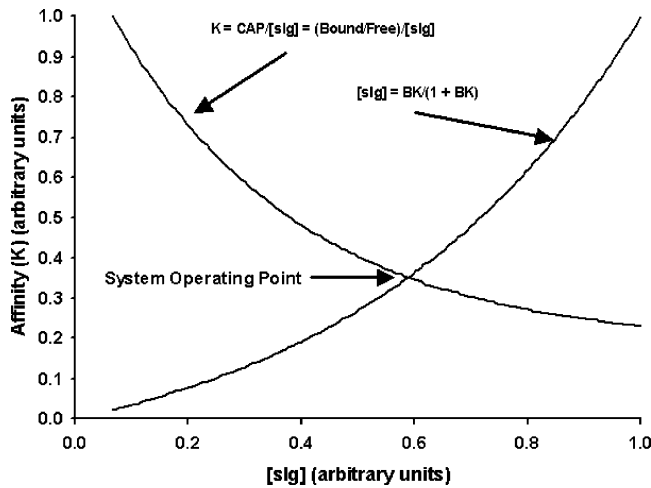


Fig. 5. Feedback system operating point. As discussed in Section 3, the optimal binding efficiency (total binding capacity) in the feedback system shown in Fig. 4 will be achieved when Eqs. (4) and (6) in Section 4 are solved simultaneously. A theoretical solution of these equations, assuming an optimal total binding capacity, is shown by the intersection of the curves.

Eq. (6):

$$G2 = \frac{d[sIg]}{dK} = \frac{\delta}{(1 + \delta K)^2} \quad (8)$$

Note that the two system gains, G1 and G2, are opposite in sign, suggesting a possible negative feedback relationship. However, the nature of the feedback relationship, if any, can only be determined empirically (Hobbie, 1997). We seek to determine how the variables of affinity (K) and concentration (sIg) change with age relative to one another.

For the variable of affinity, we have

$$\frac{dK}{dA} = \left(\frac{d}{dA} \right) \left(\frac{CAP}{[sIg]} \right) = \left(\frac{dK}{d[sIg]} \right) \left(\frac{d[sIg]}{dA} \right)$$

which, using the results from Eq. (7), gives

$$\frac{dK}{dA} = G1 \left(\frac{d[sIg]}{dA} \right) \quad (9)$$

For the variable of concentration, we have

$$\frac{d[sIg]}{dA} = \left(\frac{d}{dA} \right) \left(\frac{\delta K}{1 + \delta K} \right) = \left(\frac{d[sIg]}{dK} \right) \left(\frac{dK}{dA} \right)$$

which, using the results from Eq. (8), gives

$$\frac{d[sIg]}{dA} = G2 \left(\frac{dK}{dA} \right) \quad (10)$$

If there is a negative feedback relationship, then a theoretical plot of (dK/dA) versus $(d[sIg]/dA)$ should have a negative slope because the system gains, G1 and G2, are opposite in sign. As described previously, we followed the changes (slopes) with age, A, for these variables for each child individually (Fig. 1). Comparing these slopes against one another by correlation gave: $r = (dK/dA)/(d[sIg]/dA) = dK/d[sIg] < 0$. The theoretical and experimental results suggest a negative feedback relationship between the two independent variables

of affinity (K) and concentration ([sIg]) to achieve an optimal total binding capacity.

5. Discussion

Among children living in similar environmental conditions, including exposure to allergens, there is a ‘consolidation’ of T-cell-dependent humoral immune responses towards the most efficient production of antibodies for the specific environmental challenge, regardless of atopic status. As shown in Table 1, even in the earliest years, children are able to mount a vigorous allergen-specific humoral response reflected both by the concentrations of the antibodies produced and their binding affinities for allergen. However, based upon these results alone, there are no apparent age-dependent trends.

In contrast, upon combining the two variables for total antibody capacity (concentration $\times$ affinity) does show an age-dependent progression of humoral response changes to environmental allergen challenges. These results are in accord with previously proposed ideas regarding the possibility of “natural limits” both for Ig concentrations for a given antigen load and a ceiling for Ig affinity values.

The major component of the B-cell receptor (BCR) complex is antigen-specific, membrane-bound Ig (mIg) (Alès-Martinez et al., 1991). As a model, we can envision two competing mIg’s (mIgX1 and mIgX2), both of which require a given antigen for activation of their respective BCRs, but vary with regard to their distributions of mIg-binding affinity values. We can further propose an “affinity threshold” for mIg, where those BCRs with affinity values at or above the threshold will become activated, while those with affinity values below the threshold remain quiescent or, possibly, their B-cells become subject to death via apoptosis.

In vitro evidence has shown that there is an affinity threshold below which the BCR will not become activated (Batista and Neuberger, 1998). Further, in the presence of a limited (limiting) antigen load, the two mIg’s will vie for the antigen akin to a “selective competition” between two biological species that mutually require a particular environmental resource for their survival. Previous theoretical and experimental work following B-cell reconstitution after bone marrow irradiation in a murine model have shown how populations of B-cells with differential combining capacities for a mutually required resource will eventually achieve a homeostatic balance (Freitas et al., 1995; McClean et al., 1997).

It has also been theoretically proposed (Foote and Eisen, 1995), with support from in vitro evidence (Batista and Neuberger, 1998), that there is an “affinity ceiling” for a specific humoral response. While a minimal binding affinity by mIg is required for BCR activation, there is also a maximal mIg affinity above which there is no probable benefit for selective advantage, since most cellular properties requiring activation via ligand–receptor coupling will be activated at or below this ceiling value.

We have previously shown that allergen-specific IgE binding affinity among older children and adults with established atopic disorders is extremely high (Pierson et al., 1998; Pierson-Mullany et al., 2000, 2002; Jackola et al., 2002), and comparable to the previously proposed affinity ceiling (Foote and Eisen, 1995). Once these values were established, they remained constant throughout most of the human life span (Jackola et al., 2003). Additionally, once established, there were no significant changes in serum allergen-specific IgE concentrations with age. Thus, the total binding capacity (Ig concentration $\times$ affinity) reached a limiting value above which there was no additional benefit for the particular antigenic challenge.

With reference to Figs. 4 and 5 we can envision that, with time (age), there will also be a narrowing of the distribution of binding capacity values via a negative feedback relationship between affinity and antibody concentration. If, for example, a cell is selected with affinity, K , above that value at the optimal “operating point” for total binding capacity, then less of that sIg will need to be produced in order to obtain optimal efficiency within the system.

If the smooth curves shown in Fig. 2C are the optimal trajectories leading to the most efficient total binding capacity “operating point”, then it would appear that the immune systems of infants exposed to a non-infectious antigen tend to produce optimal amounts of antibody with regard to concentration and affinity with age. This also accounts for a decrease with age of the statistical variance of measured capacity values (Table 2).

The amount of Ig produced by a given B-cell, or B-cell-derived plasma cell, is a function of the binding affinity of the mIg incorporated in the BCR complex (Batista and Neuberger, 1998). If the optimal affinity range begins to narrow towards those values between an affinity threshold and an affinity ceiling, then the amount of Ig produced for a given antigenic challenge will also eventually reach an optimal value (concentration). As “fine-tuning” progresses via successive rounds in the negative feedback loop, smaller and smaller adjustments in the variables of K and sIg are required for each child as each of their total binding capacities is impelled towards the (presumed) optimal “operating point”. Thus, our experimental results demonstrate the dynamics of the evolution of a humoral response to a specific environmental antigen challenge in children.

We were not able to detect Der p 1-specific IgE in the cord blood samples from the atopic children. Although it has been suggested that the “atopic propensity” may result from maternal influences on the in utero environment (Prescott et al., 1998), our results show that postnatal environmental exposure to allergens is critical for the maturation and consolidation of T-cell-dependent humoral responses. Further, the developmental deviation between the atopic and non-atopic responses requires several years of exposure to allergen as shown in Fig. 2. Although a vigorous humoral response is evident in the earliest postnatal years, a clear demarcation between atopic and non-atopic status is not evident until about

4–6 years of age as previously suggested by others (Hattevig et al., 1993).

More generally, however, as shown in Figs. 2C and 3, the development of Ig responses to allergens follow similar trajectories in both atopic and non-atopic children, reflected by nearly parallel kinetics (Fig. 2C) and ‘consolidation’ of responses with age (Fig. 3). While atopic clinical conditions may be the result of an inherited predisposition to produce allergen-specific IgE, the evolution of the response is particularly well regulated. In both atopic and non-atopic children, continuous exposure to allergen (Der p) does not mimic a response that would occur upon continuous exposure to an infectious antigen. Rather, once the particular isotype involved has recognized the relatively low level of non-self antigens, there is an impetus towards stabilization of the humoral responses against these non-infectious agents. Immunologically, atopy is not an infectious disease process, but an error in isotype switching during an otherwise normal antigen recognition process.

Most importantly, our results demonstrate an immunologic regulatory mechanism via a negative feedback control system. While antigen-specific antibody concentration and binding affinity are important parameters of the humoral response, the ultimate controlling variable that defines the efficiency of the response appears to be the antibody total binding capacity. In the presence of a (presumed) constant antigen challenge, the humoral response is driven towards the most efficient combination of concentration and affinity operating via a negative feedback interaction between the two. This is another example of how separate elements of the immune system feedback upon each other to achieve an optimal response (Germain, 2001).

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