



Entropy-favored human antibody binding reactions with a non-infectious antigen

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Abstract

Little is known about the thermodynamic properties of human antibodies directed against ‘natural’ antigen surfaces, possibly due to the complex interactions that are involved. Using an affinity distribution method, we have previously characterized the binding reactions between a major allergen from ragweed, Amb a 1, and serum Amb a 1-specific IgE as a model system. We determined the temperature dependence of these interactions using serum samples from people with established allergic sensitivity to ragweed pollen. Each sample provided evidence for three epitope-specific IgE reactions with extremely high equilibrium binding affinities @ 37 °C (10^8 to 10^{11} M⁻¹). Determining the affinities over a range of temperatures (4–41 °C) revealed a favorable exothermic Gibbs free energy change, $\Delta G \approx -17.59 (\pm 5.04)$ kcal/M, comparable to previous reports using monoclonal antibodies produced against well-defined artificial antigens. In contrast to previous studies, in this system there was minimal input from enthalpy: $\Delta H \approx -2.41 (\pm 2.32)$ kcal/M. However, a significant contribution was found from entropic changes: $\Delta S \approx 48.98 (\pm 9.20)$ cal/K M. Human ‘secondary antibodies’ such as IgE, produced after exposure to ‘natural’ antigens, are optimized in terms of their high equilibrium binding constants with the antigen (allergen) that induced their production. Thermodynamically this is exemplified by minimal enthalpic (bond formation) concomitant with significant entropic (alignment) contributions to the total free energy change of reaction. These results suggest a high degree of ‘complementarity’ between the antibody and antigen surfaces in this experimental system, and may be a general guiding principle in the evolution of antibody repertoires by the adaptive immune system.

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1. Introduction

Optimal antibody binding with antigen is paramount in the development of adaptive immune responses. Knowledge of the biophysical properties of antigen–antibody interactions is essential for understanding antibody repertoire development during the evolution of an adaptive immune response. Akin to other protein–protein associations, antigen–antibody complexes arise from non-covalent chemical bonds and molecular interactions including electrostatic and van der Waals forces, hydrogen bonds and hydrophobic effects (Clothia and Janin, 1975; Ross and Subramanian, 1981; Mariuzza et al., 1994).

Despite these relatively transient associations, mammalian antibodies are able to achieve a high level of specificity for antigens reflected by high equilibrium binding constants (Foote and Eisen, 1995). High equilibrium binding affinities with antigen reflect favorable total Gibbs free energy changes (ΔG) for these reactions. Yet, the question arises of what thermodynamic forces direct these processes to achieve these levels of specificity?

Previous studies to establish the relative enthalpic (ΔH) and entropic (ΔS) contributions of antigen–antibody reactions have mostly relied upon ‘simple’ experimental systems with monoclonal antibodies raised against artificial antigens like hen egg lysozyme (HEL) or small molecular weight haptens. Designed primarily to elaborate upon the intermolecular forces of antibodies directed against single epitopes, these experimental systems exploit the detailed information derived from isothermal titration calorimetry (Yasui et al., 1998; VanAntwerp and Wittrup, 1998)

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or binding association and dissociation kinetics via biosensors (Zeder-Lutz et al., 1997; Sagawa et al., 2003). In these and similar reports it has been found that enthalpic contributions appear to be the ‘dominant’ force driving the total free energy changes. These systems require detailed *a priori* structural and chemical kinetic information in order to place the derived thermodynamic information into a proper modeling context.

Mammalian antibody repertoires develop in the germinal centers of secondary lymph nodes (Wabl and Steinberg, 1996). Via the complementary activities of isotype class switch recombination and somatic hypermutation (SH) an antibody repertoire is achieved that is ‘biologically optimal’ for a particular antigenic challenge. Due to affinity maturation in germinal centers, ‘secondary antibodies’ like IgG or IgE have significantly higher binding constants for antigens than do ‘primary’ IgM antibodies (Hodgkin, 1997). But, due to the highly stochastic nature of the events involved with SH within germinal centers, the thermodynamic characteristics have not been established to any degree of certainty.

Allergens are common sources of non-infectious antigens that may consist of multiple immunogenic epitopes (Blumenthal and Rosenberg, 1999). We have devised a methodology using purified major allergens and antibodies produced by humans against these allergens isolated from serum. This method does not rely upon *a priori* knowledge regarding antibody interactions with ‘natural’ antigen surfaces (Pierson et al., 1998). Rather, it details the number of epitope-specific binding reactions that occur and their thermodynamic characteristics reflected by most probable equilibrium binding constants.

We studied the temperature dependence of these binding reactions, which characterizes the forces contributing to favorable total free energy changes; enthalpic energy changes involved with heat of bond formation and entropic changes reflecting the degree of ‘complimentarity’ or alignment between the two surfaces. We demonstrate that entropic rather than enthalpic contributions are the dominant forces contributing to favorable total free energy changes of human antibodies reacting with ‘natural’ antigen surfaces.

2. Methods

2.1. Study population

As part of ongoing studies of human atopic diseases, we have recruited people from the Asthma and Allergy Clinic, Fairview-University Medical Center, Minneapolis, MN with history of these disorders and have stored banks of frozen serum (-80°C) for additional analyses. We randomly selected serum samples from 10 individuals who had established allergic sensitivity to short ragweed pollen (Amb a; *Ambrosia artemisiifolia*). Subjects ranged in age from 14 to 54 years (4 females, 6 males). Evidence for allergic sensitivity to ragweed was established by a positive result for standardized percutaneous skin prick test (SPT) using Amb a allergen extract. An SPT [+] result provides evidence for the presence of allergen-specific IgE that is of extremely high binding affinity for an allergen (Pierson-Mullany et al., 2002). All donors provided written, informed consent according

to the guidelines of the Institutional Review Board, University of Minnesota Medical School.

2.2. Amb a 1-Specific IgE

Quantitative assays for allergen-specific IgE were done using the purified allergen Amb a 1, a major allergen extracted from short ragweed (*Ambrosia artemisiifolia*). Amb a 1 (m.w. 37,800) was purified and biotinylated as described (Pierson et al., 1998). Serum concentrations of Amb a 1-specific IgE were measured by an adaptation of the Access[®] (Beckman Coulter, Chaska, MN) specific IgE immunoassay (Pierson et al., 1998; Allauzen et al., 1999; Pierson-Mullany et al., 2000). The lowest limit of detection is 0.02 IU/ml (1 IU = 2.4 ng)

2.3. Binding affinity distribution functions (automated method)

Our basic experimental method provides information regarding allergen-antibody (Ag-Ab) binding reactions in vitro (Pierson et al., 1998; Pierson-Mullany et al., 2000). Briefly, magnetic particles (Dynal, Lake Success, NY) used in the Access[®] system are coated with a mouse anti-human, anti-IgE mAb. To this is added a serum sample known to contain allergen-specific IgE, which is “captured” by the particle-bound mAb. Biotinylated, purified allergen (BT-Amb a (1) is added to bind only to the specific IgE, and detection is achieved by the addition of streptavidin-conjugated alkaline phosphatase (Sigma, St. Louis, MO) and a chemiluminescent substrate (Lumi-phos 530[®], Lumigen, Inc., Southfield, MI) to generate a light signal.

Using a range of purified Ag (BT-Amb a 1) concentrations, a binding isotherm is generated: r vs. $\log_{10} [\text{Ag}]$, where r = 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. 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; Pierson-Mullany et al., 2000) 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. This function identifies 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 ($p[K_A]$). The data were analyzed using Mathematica[®] software (Wolfram Research, Inc., Champaign, IL).

The strength of the affinity distribution method is the capability to detect multiple reactions that may occur simultaneously,

which is the case with purified allergens that may consist of multiple allergenic epitopes. Multiple peaks represent responses by different antibody clones to these epitopes (Pierson-Mullany et al., 2000).

2.4. Temperature dependence of affinity distributions (manual method)

Although the automated Access[®] system provides detailed analyses of antigen–antibody reactions, by manufacturer's specifications information is only available @ 37 °C. To determine the affinity distributions over a range of temperatures, we adapted the Access[®] methodology using magnetic beads (Dynal) as a solid phase to a 96 well microtest plate format (Costar 3912, Corning, NY). All steps were as described above, except that the reactions were allowed to occur in microtest plates that were kept in temperature-regulated water baths (4, 25, 30, 37 and 41 °C). Prior to the start of each assay, all reagents and equipment were allowed to equilibrate to the respective temperatures for a minimum of one hour. Removal of excess reagents after each experimental step was achieved by separation of the magnetic particles using a BioMag[®] 96-well plate separator (Perseptive Biosystems, Framingham, MA) and five washes with buffer. At the last experimental step, chemiluminescent substrate was added to the test wells and chemiluminescent light signals were read with a TR717 Microplate Luminometer (Tropix, Inc., Bedford, MA).

2.5. Thermodynamic relationships and data analysis

The Gibb's free energy change (ΔG : kcal/M) of a reaction is related to the enthalpy change (ΔH : kcal/M) and the entropy change (ΔS : cal/K M) by the relation.

$$\Delta G = \Delta H - T\Delta S \quad (T = \text{absolute temperature (K)}) \quad (1)$$

The Gibb's free energy change is also related to the equilibrium association constant (K_A) by the equation

$$\Delta G = -RT \ln [K_A], \quad (2)$$

where R is the universal gas constant (1.987 cal/K M) and $\ln$ is the natural logarithm.

Comparing Eqs. (1) and (2)

$$-RT \ln [K_A] = \Delta H - T\Delta S. \quad (3)$$

Rearranging Eq. (3) gives

$$\ln [K_A] = -\frac{\Delta H}{RT} + \Delta \frac{S}{R} \quad (4)$$

Plotting $\ln [K_A]$ vs. $1/T$ yields a straight line with slope = $-\Delta H/R$ and intercept = $\Delta S/R$.

These relationships were analyzed by simple linear regression, with 'goodness of fit' assessed by analysis of variance (ANOVA), using statistical analysis software provided by Mathematica[®] (Wolfram) and Microsoft Excel[®] (Redmond, WA).

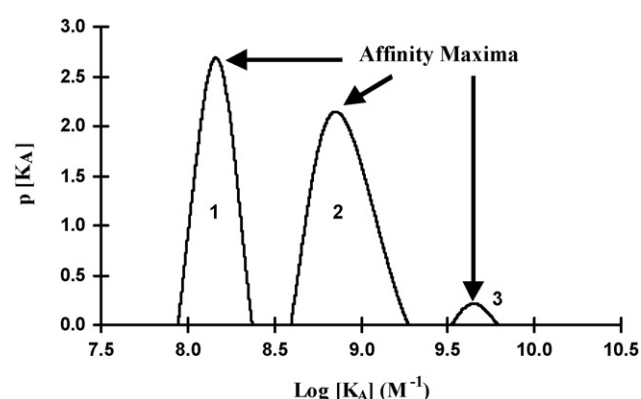


Fig. 1. Representative affinity distribution. The three primary features are the number of reactions involved between Amb a 1 allergen and IgE antibodies, the relative contribution, $p[K_A]$ of each reaction reflected by the height of the affinity maxima (indicated by arrows) and the affinity constant associated with each affinity maximum.

3. Results

3.1. Comparisons of affinity distribution determination methods

We determined the equilibrium binding characteristics between a purified major allergen from ragweed, Amb a 1, and Amb a 1-specific IgE from serum samples obtained from 10 people with allergic sensitivity to ragweed pollen residing in the Minneapolis/St. Paul, MN metropolitan area. Using the automated Access Immunoassay System[®], Fig. 1 shows a typical affinity distribution for the reactions between an allergen-specific immunoglobulin and the purified allergen Amb a 1 @ 37 °C. The three key features in this figure are the number of reactions involved (n), the most likely affinity constant (K_A) for each reaction (highest point of each 'affinity peak') and the relative proportion ($p[K_A]$) of each reaction (height of each peak relative to the other peaks).

Table 1
Comparison of methods for affinity distribution determinations

	Manual ^a	Automated ^a	<i>t</i> (d.f.) ^b	<i>p</i> -Value
Low affinity (Rxn. 1) ^c				
Mean	8.31 ^d	8.70	2.15 (95)	0.025
Standard deviation	0.35	0.56		
<i>N</i>	10	87		
Intermediate affinity (Rxn. 2)				
Mean	9.04	9.20	0.71 (95)	N.S. ^e
Standard deviation	0.49	0.69		
<i>N</i>	10	87		
High affinity (Rxn. 3)				
Mean	9.90	9.90	0.005 (61)	N.S.
Standard deviation	0.69	0.61		
<i>N</i>	10	53		

^a Manual and automated methods described in Section 2.

^b T -test comparing mean values for manual and automated methods.

^c Reaction (Rxn) numbers refer to Fig. 1.

^d Values are base-10 logarithms.

^e N.S. = not significant @ $p = 0.05$.

By manufacturer-established settings, information from the Access[®] analyzer is limited to determinations @ 37 °C (Automated Method). As described in Section 2, in order to determine the characteristics of these affinity distributions at different temperatures, we adapted the Access[®] methodology to microtest plate formats (Manual Method). Table 1 compares the results for the samples analyzed in this study by this adapted method @ 37 °C to results we have previously reported for serum samples obtained from ragweed sensitized individuals using the automated Access[®] method (see Table 1 in Pierson-Mullany et al., 2000).

Using the Manual Method, each of the ten samples assayed provided evidence for three presumptive epitope-specific IgE reactions. As shown in Table 1, the Manual Method provided affinity distribution information comparable to the Automated Method @ 37 °C.

3.2. Thermodynamic characteristics of the affinity distributions

Using the Manual Method, we determined the affinity distributions for the Amb a 1-IgE binding reactions at five temperatures (4, 25, 30, 37 and 41 °C). Fig. 2 shows a representative example of these results with the natural logarithm of the association constant for each epitope-specific reaction, $\text{Ln}[K_A]$, plotted against the inverse of the reaction system temperature, $1/T$, in K. By the relationship shown in Eq. (4) of Section 2 ($\text{Ln}[K_A] = -\Delta H/RT + \Delta S/R$), this plot should yield a straight line with slope = $-\Delta H/R$ and intercept = $\Delta S/R$. The values for enthalpy changes (ΔH) and entropy changes (ΔS) shown in Fig. 2 were obtained by simple linear regression based upon this relationship.

Table 2 summarizes the results for the ten samples tested. As shown in Fig. 2, estimated values for enthalpy (ΔH) and entropy (ΔS) were determined from regression analyses of the experimental data. In all cases, a significant linear relationship was found as assessed by ANOVA with $p \leq 0.05$. From

Table 2

Summary of thermodynamic results (37 °C)

Rxn. #1 (lowest affinity)				
dDonor	Log ₁₀ [K]	ΔG (kcal/M) ^a	ΔH (kcal/M) ^a	ΔS (cal/K M) ^a
1	8.00	−7.34	−1.11	20.10
2	8.83	−14.85	−1.86	41.90
3	8.29	−13.12	−1.81	36.47
4	8.45	−31.16	−9.90	68.58
5	8.70	−15.34	−1.98	43.09
6	9.20	−24.58	−5.24	62.40
7	8.75	7.03	8.92	6.11
8	8.42	−20.46	−3.10	56.00
9	8.70	−15.34	−1.22	45.56
10	8.82	−18.21	−2.49	50.70
Mean	8.70	−15.34	−1.98	43.09
S.E.M	0.106	3.231	1.472	5.958
Rxn. #2 (intermediate affinity)				
1	8.31	−13.85	−2.11	37.88
2	9.20	−19.96	−4.03	51.77
3	8.88	−11.79	0.52	39.71
4	8.96	−30.57	−9.04	69.44
5	9.26	−20.10	−3.60	53.22
6	10.01	−31.58	−8.84	73.35
7	9.89	2.50	7.44	15.95
8	8.79	−14.29	−1.51	41.22
9	9.32	−22.83	−4.04	60.61
10	9.29	−23.35	−4.03	62.32
Mean	9.20	−18.58	−2.92	50.55
S.E.M	0.159	3.143	1.485	5.464
Rxn. #3 (highest affinity)				
1	8.79	−16.91	−2.12	47.71
2	9.63	−20.05	−3.61	53.02
3	9.62	−20.26	−3.31	54.68
4	9.97	−4.46	4.84	29.99
5	10.76	−25.90	−5.32	66.38
6	10.78	−23.56	−4.22	62.40
7	10.48	−18.84	−2.32	53.29
8	8.91	−22.78	−3.51	62.17
9	10.03	−19.44	−2.67	54.11
10	10.02	−16.24	−0.99	49.18
Mean	9.90	−18.84	−2.32	53.29
S.E.M	0.217	1.852	0.881	3.209

^a Estimates for ΔH and ΔS were found by simple linear regression using Eq. (4) in Section 2. From these results, estimates for ΔG were found using Eq. (1) in Section 2.

these estimates, total Gibbs's free energy changes (ΔG) were determined from the relationship shown in Eq. (1) in Section 2 ($\Delta G = \Delta H - T\Delta S$) @ $T = 37$ °C.

As expected, the equilibrium binding constants for the three reactions were different for each of the samples assayed (shown as $\log_{10} [K]$ values in Table 2). However, the estimates for the three thermodynamic parameters were remarkably similar. We compared the average values of these estimates for the three different reactions using one-way ANOVA. Total Gibbs free energy changes (ΔG): $F(2, 27) = 0.48$; $p = 0.62$. Enthalpy changes (ΔH): $F(2, 27) = 0.13$; $p = 0.88$. Entropy changes (ΔS): $F(2, 27) = 1.11$; $p = 0.35$.

As we have previously shown (Pierson-Mullany et al., 2000), each of the 'affinity peaks' in Fig. 1 represents IgE produced against a unique immunogenic surface (presumptive epitopes)

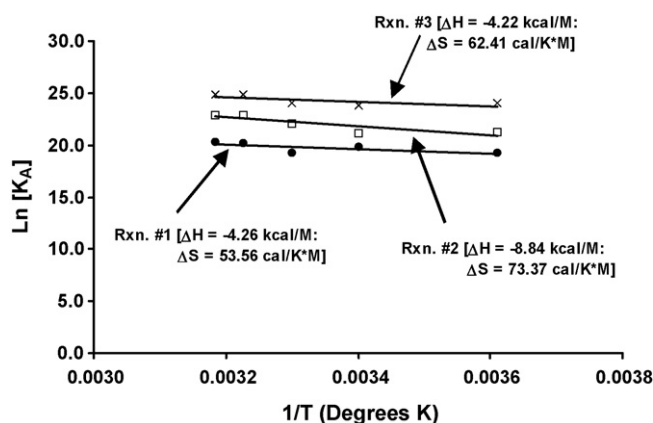


Fig. 2. Representative temperature dependence of binding reactions. The natural logarithm of the affinity constant, $\text{Ln}[K_A]$, for each reaction (see Fig. 1) is plotted against the inverse of the temperature, $1/T$ (K). Estimates for enthalpy (ΔH) and entropy (ΔS) changes were found by simple linear regression using Eq. (4) in Section 2.

found on the major ragweed allergen Amb a 1. The major difference in these epitope-specific reactions is their equilibrium binding constants. Yet, as shown by the ANOVA results above, their thermodynamic characteristics are similar. Of particular note, estimates for enthalpy changes (ΔH) provide minimal contribution to the changes of total free energy (ΔG ; Table 2).

3.3. Entropy–enthalpy relationship comparisons

As shown in the Section 3.2, each of the serum samples assayed provided evidence for IgE produced against three specific antigenic surfaces (presumptive epitopes). On average, aside from differences in equilibrium binding constants, the thermodynamic parameters associated with total free energy changes were similar for these three reactions. To determine if this was a common thermodynamic phenomenon related to these binding reactions and not due to “averaging out” in the statistical analysis, we compared the relationships between the estimates for entropy (ΔS) and enthalpy (ΔH) changes.

Eq. (4) in Section 2 is: $\text{Ln}[K_A] = -\Delta H/RT + \Delta S/R$. To eliminate the effect of variable association binding constants, we rearranged this equation to

$$\Delta \frac{S}{R} - \text{Ln}[K_A] = \frac{\Delta H}{RT} \quad (5)$$

If the entropy–enthalpy relationships are the same for all three reactions, a plot of $\Delta S/R - \text{Ln}[K_A]$ vs. ΔH should yield a straight line with zero intercept. These results are shown in Fig. 3.

If the entropy–enthalpy relationships were different for the three reactions, we might observe significant curvilinearity in the plotted results. However, from simple linear regression: $\Delta S/R - \text{Ln}[K_A] = 0.93 (\pm 0.58) - 1.79 (\pm 0.12) \times \Delta H$; $F(1, 28) = 207.2$; $p < 10^{-14}$.

The slope of this line was significantly different from zero: $t(29) = 14.4$; $p < 10^{-14}$. However, the intercept value was not significantly different from zero: $t(29) = 1.61$; $p = 0.12$.

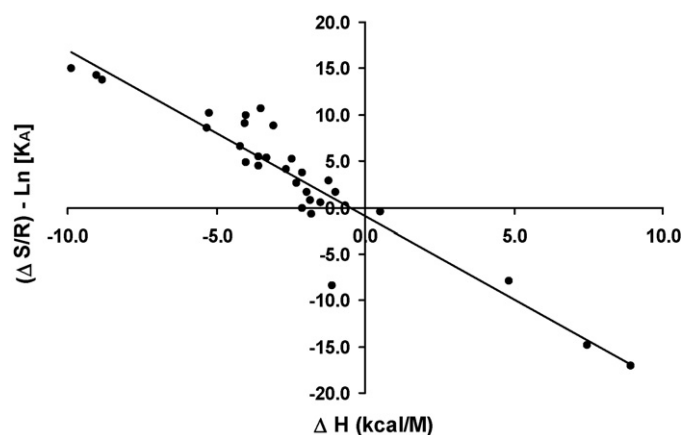


Fig. 3. Entropy–enthalpy relationships. Using Eq. (5) in Section 3, values for $(\Delta S/R) - \text{Ln}[K_A]$ are plotted against ΔH . Straight line is best-fit result of linear regression.

Thus, the thermodynamic characteristics for changes in total free energy for the three separate epitope-specific IgE reactions are similar.

From the results in Table 2, the overall average values ($\pm$ S.E.M.) for the thermodynamic parameters are summarized as follows (total number of reactions = 30): $\Delta G \approx -17.59 (\pm 5.04)$ kcal/M; $\Delta H \approx -2.41 (\pm 2.32)$ kcal/M; $\Delta S \approx 48.98 (\pm 9.20)$ cal/K M. For the reactions of human IgE antibodies directed against ‘natural’ antigen surfaces, favorable total free energy changes of reaction (ΔG) are comprised of minimal enthalpic changes (ΔH) and significant entropic changes (ΔS).

4. Discussion

Previous studies to establish the relative enthalpic and entropic contributions of antigen–antibody reactions have mostly relied upon ‘simple’ experimental systems with monoclonal antibodies raised against artificial antigens like hen egg lysozyme (HEL) or small molecular weight haptens. Designed primarily to elaborate upon the intermolecular forces of antibody reactions with single antigenic epitopes, these experimental systems were chosen to exploit the detailed information that can be derived from isothermal titration calorimetry (Yasui et al., 1998; VanAntwerp and Wittrup, 1998) or binding association and dissociation kinetics via biosensors (Zeder-Lutz et al., 1997; Sagawa et al., 2003). In these and similar reports, it has been found that enthalpic contributions appear to be the ‘dominant’ force driving the total free energy changes.

It has also been found in these ‘simple’ systems that antibodies that are more “affinity matured” also have a significant entropic contribution, suggesting that affinity maturation of antibodies leads to a more energetically favorable alignment between protein–protein surfaces (Sagawa et al., 2003). However, these experimental systems must rely upon detailed *a priori* knowledge of the structural components and their binding reactions; otherwise the derived information cannot be placed into a proper modeling context. Whether or not these thermodynamic relationships hold for human antibodies directed against ‘natural’ antigen surfaces has not been established (Hodgkin, 1997).

The primary ‘purpose’ of antibodies is to bind to antigens and clear them from the system (Steward, 1981). As antibody formation arises at the expense of metabolic energy, the most thermodynamically efficient processes will have an evolutionarily selective advantage. However, as the adaptive immune systems of mammals, including humans, must deal with a virtually infinite array of antigenic challenges, a key problem arises in that the immune system must first ‘learn’ about the nature of the challenge before it can mount an efficient response and develop ‘memory’ about the challenge for subsequent encounters (Rajewsky, 1996).

As with other protein–protein associations, antigen–antibody complexes arise from non-covalent chemical bonds and molecular interactions including electrostatic and van der Waals forces, hydrogen bonds and hydrophobic effects (Clothia and Janin, 1975; Ross and Subramanian, 1981; Mariuzza et al., 1994). These are ‘weak’ associations that occur over relatively ‘short’

intermolecular distances. Thus, processes of ‘learning’ and ‘memory’ formation that favor optimal alignments between opposing protein surfaces may provide a selective advantage to ‘clones’ of cells and their antibody products.

Yet, with the possible exception of the toll-like receptors of the innate immune system, those that recognize antigen surfaces that are more ‘ancient’ on an evolutionary scale, the adaptive immune system has no *a priori* knowledge of what surfaces it may encounter; the germline is naïve regarding what instructions it must follow when encountering most ‘natural’ antigen surfaces. Thus, akin to Darwinian evolution on a microscopic scale, the adaptive immune system attempts to cover all contingencies by initially producing a wide array of antibody surfaces followed by selecting those best fit to meet the given challenge.

Antibody repertoire development and clonal selection occurs within the germinal centers of secondary lymph nodes (Lindhout et al., 1997; Tarlinton and Smith, 2000). Repertoire development arises from the complementary activities of isotype class switch recombination and somatic hypermutation, which leads to affinity maturation (Wabl and Steinberg, 1996). Initially IgM-bearing and secreting B cells are replaced by B cell clones that produce one of the ‘secondary antibodies’ (IgA, IgG, IgE) that are better adapted for a particular challenge. Those best adapted are also selected for survival via their B cell receptor (BCR), a major component of which is membrane-bound immunoglobulin (mIg).

Those B cells with the highest affinity mIg-BCR’s are the most likely to survive, while other clones with lower affinity mIg will die via apoptosis. Antibody repertoire development favors the highest affinity antibodies (Foote and Eisen, 1995; Rajewsky, 1996). Further, a slower disassociation rate by the mIg for an antigen, corresponding to longer antigen ‘retention time’ by the BCR, favors prolonged B cell activation (Batista and Neuberger, 1998). Thus, thermodynamic forces associated with enhancing higher binding affinity antibodies, primarily by promoting slower dissociation rates, will provide the selective forces for repertoire development.

We have previously shown that people with established atopic disorders, such as allergic sensitivity to common non-infectious allergens, will produce IgE specific for these allergens that is of extremely high equilibrium binding affinity (Pierson et al., 1998; Pierson-Mullany et al., 2000, 2002). Indeed, these antibodies may be at an upper limit of biologically meaningful affinities produced by humans (Foote and Eisen, 1995). These experimental results derived from humans who had been exposed to allergens for many years.

However, production of high affinity antibodies against common aeroallergens is not restricted to those with atopic disorders (Jackola et al., 2002), or to those people exposed to allergens for many years of their lives (Jackola et al., 2005). Allergens, like ragweed pollen or house dust mites, are ubiquitously distributed non-infectious antigens to which people will produce high binding affinity antibodies of the ‘secondary isotypes’ (primarily IgE or IgG1). Thus, they provide an ideal model for elaborating the thermodynamic characteristics of antibody repertoire development in humans after ‘natural exposures’ to ‘natural antigen surfaces.’

A continuation of our previous results is the study of the temperature dependence of allergen–antibody reactions, as these reveal the major thermodynamic characteristics of the high affinity antibodies. Unlike the previously cited ‘simple’ systems that have used monoclonal antibodies produced against artificial antigens, our experimental system requires no *a priori* knowledge of the nature of these reactions (Pierson et al., 1998). Rather, the method elaborates the number and binding affinities of the antibodies found in human serum produced against allergens (presumptive epitopes on the major ragweed allergen Amb a 1; Fig. 1).

Comparable to results using monoclonal antibodies (Zeder-Lutz et al., 1997; Yasui et al., 1998; VanAntwerp and Wittrup, 1998; Sagawa et al., 2003), the high affinity reactions of human IgE against allergenic surfaces shows extremely favorable, exothermic changes in total free energy (ΔG ; Table 2). But, in contrast to most results with ‘simple’ systems, this resulted with a minimal contribution from enthalpic changes (ΔH) associated with bond formation (Fig. 2). The major contribution to the total free energy change arose from entropic changes (ΔS).

Monoclonal antibodies that are more “affinity matured,” either derived from hybridoma technology (Sagawa et al., 2003) or isolated from ascites fluid (VanAntwerp and Wittrup, 1998), also show a significant entropic contribution compared to lower affinity monoclonals directed against the same artificial antigens. The entropic contribution to the higher affinity products was not accompanied by a significant change in enthalpy, suggesting that artificial affinity maturation arises from enhancing the alignment or ‘complimentarity’ of the antibodies for the antigen binding surfaces (Hodgkin, 1997). However, in these cases, although the enhanced antibody affinity arose due to an apparent increase in ‘induced fit,’ the enthalpic change was still the major thermodynamic contribution to the total free energy changes of the binding reactions, accounting for 80% or more of the total free energy changes (Sagawa et al., 2003).

In our experimental system, with comparable exothermic changes of total free energy, enthalpy does not make as significant contribution as that seen in the ‘simple’ systems, accounting for 15% or less of the total free energy changes (Table 2). Of course, the IgE antibodies found in human serum samples undoubtedly derived from prolonged ‘selection’ processes within lymph node germinal centers that cannot be taken into account. Yet, the final products may be reflective of ‘total selective pressures’ both intrinsic and extrinsic to the mechanics of somatic hypermutation and affinity maturation.

The intrinsic factors contribute to the selective mutation of the genes encoding for the variable heavy and light antibody chains leading to a better induced fit between opposing antigen–antibody surfaces. However, these mechanisms must operate within an *in vivo* physiological context that must be homeostatically favorable for all metabolic processes, and not just those favorable to a particular mechanism of interest; factors extrinsic to those of the particular mechanism also bear upon the total energy balance.

By definition, exothermic binding reactions, although desirable for efficient processing of antigens, result in the release of heat that is available to do useful work somewhere else within

the total system. Regardless of the nature of this work, this heat will be absorbed. In simplest terms, the amount of heat absorbed by the system, or heat capacity (C_p), is related to the enthalpy and temperature by: $C_p = \Delta H / \Delta T$ (Atkins, 1982). Any exothermic reaction will lead to an increase in absorbed heat somewhere in the physiological system.

Temperature regulation is a major factor contributing to homeostasis in mammalian systems; the sum of all metabolic activity must be maintained within a very narrow temperature range. Intrinsic processes that can simultaneously produce their desired effect and contribute to overall homeostasis will, with highest probability, be selected at the expense of those that are less efficient at meeting these complementary demands. In this context, antibodies that can evolve to high binding affinity without unduly contributing to the overall heat content within the total system (entropy-favored) will probably be selected over those that require significant heat of formation (enthalpy-favored).

In the physiological system reported here, it would appear that entropy-derived affinity maturation, with minimal contribution from enthalpy, is favored by affinity matured ‘secondary antibodies’ produced by humans against ‘natural antigen surfaces.’

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