Development of immunotherapeutics and immunization regimes

Characterization of serum antibody responses during development of an anti-IgE based treatment for allergy and asthma, using Biacore T100

- Rapid, high information content characterization of immune responses direct from rat and primate serum
  - serum response patterns monitored over time following multiple immunizations
  - IgG subclass rapidly determined
  - qualitative changes derived from kinetic profiles
- Serum response assays correlated well with observed biological effects
- Significant advantages shown over ELISA
  - higher quality data: much lower inter- and intra-assay variations
  - higher information content: kinetic profiling of antibody stability
  - earlier detection of immune responses
- Invaluable information acquired for development of an immunotherapeutic approach:
  - minimum dose and number of administrations required for optimal stability of antibody: antigen complex
  - affinity maturation of antibody responses

"I strongly believe that this application of protein interaction analysis could play a very important role in preclinical and clinical research programs for novel immunotherapies and vaccines, enabling better treatment regimes for patients and reduced cost of goods for pharmaceutical companies"

Stefan Persson, PhD, Vice-President, Safety Pharmacology and Toxicology, Resistentia Pharmaceuticals AB

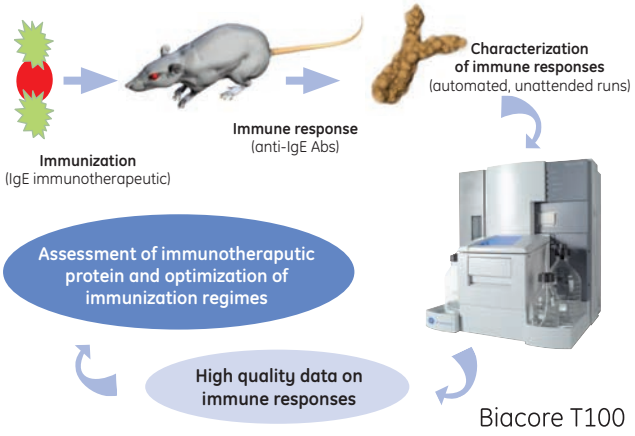

Figure 1. The role of Biacore T100 in the development of an immunotherapeutic protein against IgE-mediated diseases (allergy and asthma).

Introduction

The growing role of biopharmaceuticals in human medicine has placed increasing importance on the ability to characterize immune responses during pre-clinical development and clinical trials. This information is vital in order to design treatments that use the lowest possible drug doses, thereby minimizing both the risk of unspecific toxicological responses in patients and the cost of goods for pharmaceutical companies. It is also important to develop dosing regimes that use as few administrations as possible, in order to improve patient compliance and provide an advantage over competing therapies.

Acknowledgement

We gratefully acknowledge Resistentia Pharmaceuticals AB for providing serum samples and reagents and would like to thank Stefan Persson and Michael Fant for their invaluable contributions during this collaboration.

While ELISA has been the traditional technique used to study serum antibody responses, protein interaction analysis using Biacore™ systems has increasingly demonstrated its value in this area. In clinical immunogenicity applications during biopharmaceutical development for example, several groups have reported critical advantages in using Biacore systems compared to ELISA[1, 2].

Purpose of study

Serum antibody responses play a central role in the development of vaccines and immunotherapeutic proteins. Development can be a long and complex process, requiring the optimization of vaccine variants and immunization regimes, characterization of serum responses over extended time periods and assessment of the biological effectiveness of the antibodies produced. This procedure also involves progression through one or more test species prior to human trials. In collaboration with Resistentia Pharmaceuticals AB (Uppsala, Sweden), Biacore T100 was used to characterize immune responses in a study aimed at developing a general immunotherapeutic approach against atopic allergies and asthma.

ALLERGY & THE ROLE OF IgE

Allergy represents a major and growing medical problem, with around 20% of the general population affected to some degree. Immunoglobulin E (IgE) is the key protein involved in allergic responses, signaling the presence of allergens via high affinity IgE receptors on the surface of mast cells. Allergen-induced cross-linking of IgE to the receptor causes degranulation of the mast cells and the release of multiple substances (e.g. histamine) responsible for the clinical effects of allergic responses.

The central role of IgE in allergic responses has prompted studies suggesting that a recombinant IgE-derived immunotherapeutic protein could revolutionize the treatment of allergy and associated problems such as asthma. In this strategy (analogous to a vaccine approach against pathogenic infections), patients are induced to produce antibodies against their own IgE, thereby blocking normal allergic responses. Previous studies have established the potential of this approach to significantly reduce serum IgE levels and allergic responses in immunized animals[3]. The immunotherapy is reversible, allowing IgE levels to be reestablished if required[3].

The recombinant immunotherapeutic proteins include three IgE domains, of which the central (receptor-binding) domain is derived from the species to be immunized and the flanking domains are derived from an evolutionarily distant mammal. This structural arrangement is designed to maximize the immune response against circulating IgE, while minimizing the risks of inducing receptor cross-linking.

Results from two species, rats and Cynomolgus monkeys, are reported here. In the rat studies, serum antibody responses were characterized over time (response levels and IgG subclass) and were also correlated with changes in circulating IgE levels in the immunized animals. In the primate studies, unique real-time binding analysis enabled a kinetic assessment of the quality of serum responses over time. Direct comparisons of the results with equivalent ELISAs were carried out in order to assess the new approach with previous assay methods.

Results

Monitoring the immune response

A commercially available full-length rat IgE protein was immobilized on the sensor surface and used to detect anti-IgE Abs in serum samples (see Figure 8A). Preliminary experiments established optimal conditions for the study, including an assay temperature of 37°C and the addition of carboxymethylated dextran to the sample buffer to minimize non-specific binding of serum components to the sensor surface. Data were derived as real-time plots of binding response against time (sensorgrams) over the whole course of an interaction. In these studies, a single response level was selected at a specific time point from each sensorgram to define anti-IgE responses for each serum sample (Figure 8C).

Similar immune response patterns in rats using two adjuvants

Rats were injected with a recombinant protein (ORO) composed of a rat C3 domain (constant domain containing the IgE receptor-binding region) flanked by opossum C domains. Two different adjuvants were also used in these studies, referred to as Adj-1 and Adj-2.

Figure 2 shows the immune responses for 10 immunized and 10 control animals (using Adj-1 as adjuvant), with serum samples taken at regular intervals over the course of 3 months. The responses of the immunized animals were readily distinguished from the control samples in most cases, with considerable variations in the magnitude and temporal pattern of response observed. A number of animals showed a peak immune response at around 5 weeks (around the time of the third of four immunizations) followed by a decline, whereas others peaked at between 7 to 12 weeks. In three cases, the immune responses continued to show a rising trend at the end of the 12-week period.

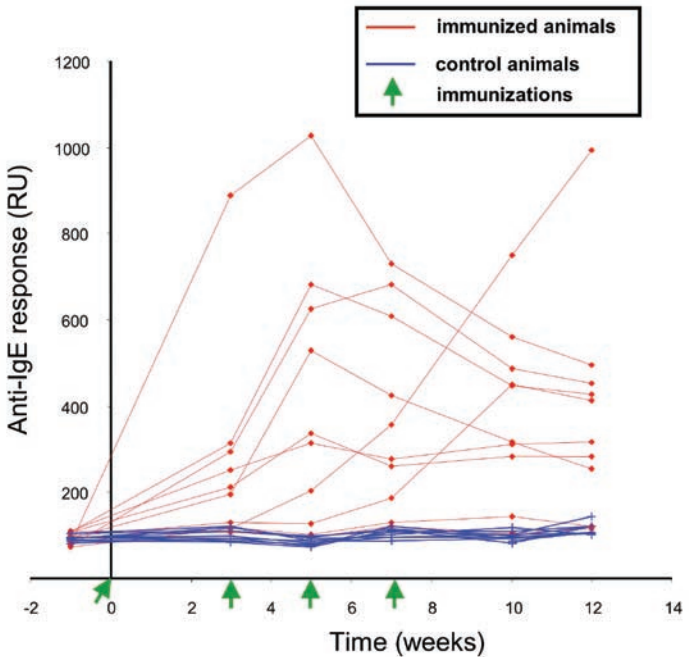

Figure 2. Rat serum responses to multiple immunizations with the immunotherapeutic protein using Adj-1. The anti-IgE response (*i.e.* serum Ab level) was measured in terms of binding response (in resonance units, RU) to full-length rat IgE immobilized on the sensor surface.

In the parallel study using Adj-2 as adjuvant, the general range of response patterns observed was similar (Figure 3), although the average response levels were only around 50% of those seen with Adj-1.

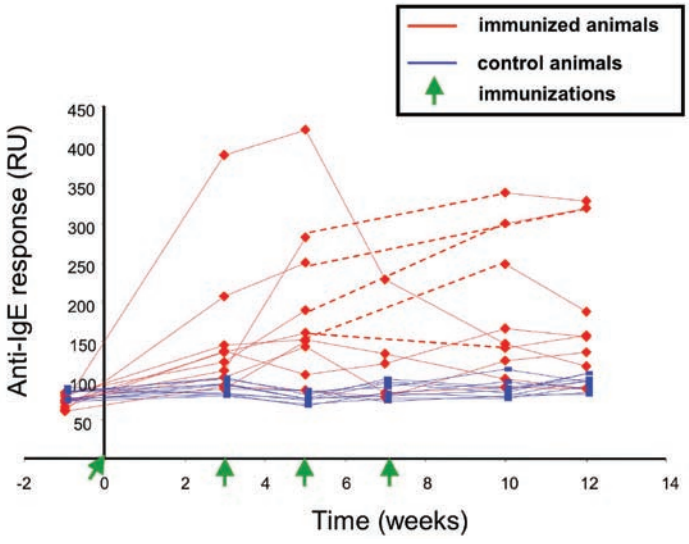

Figure 3. Rat serum responses to multiple immunizations with the immunotherapeutic protein using Adj-2. The broken red lines indicate instances where specific time point samples at week 7 were missing for 5 of the immunized animals.

Good correlation with ELISA

Results from the Biacore T100 anti-IgE serum antibody assay were directly compared with those from standard ELISAs run on the same samples at Resistentia. The immune response patterns were very similar for individual animals over the course of the 13-week study (Figure 4 B & C). Comparison of the total data sets (Figure 4A) showed a good correlation coefficient (0.87); with the Adj-1 adjuvant samples exhibiting a somewhat better correlation (0.90) than the Adj-2 samples (0.79). Good correlation was independent of the type of response pattern exhibited by individual animals. Figure 4B shows the correlation plot for animal 1039, which exhibits the most common response pattern, with a peak at around 7 weeks and a subsequent drop. A similarly close correlation can also be seen for animal 1038 (Figure 4C), which exhibits a quite different, continuously increasing response pattern. A good correlation was seen for the large majority of all the individual animals in the study, but Figure 4D shows one interesting exception in which an anti-IgE response was detected by the Biacore T100 assay before it was detectable by ELISA. Similar observations have been made previously in clinical immunogenicity applications[2] and this was also seen more prominently in the primate studies here.

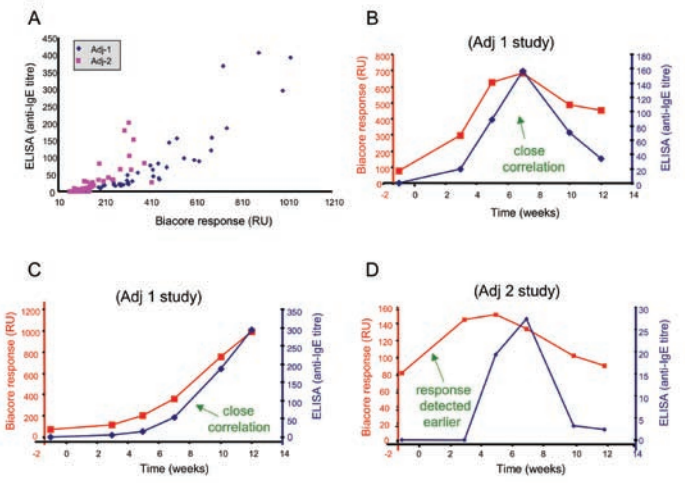

Figure 4. Comparison of rat anti-IgE serum responses using Biacore T100 and ELISA. (A) ELISA antibody titers are plotted against interaction analysis responses for the complete data sets from the Adj-1 and Adj-2 adjuvance studies. (B-D) Correlation plots for individual animals.

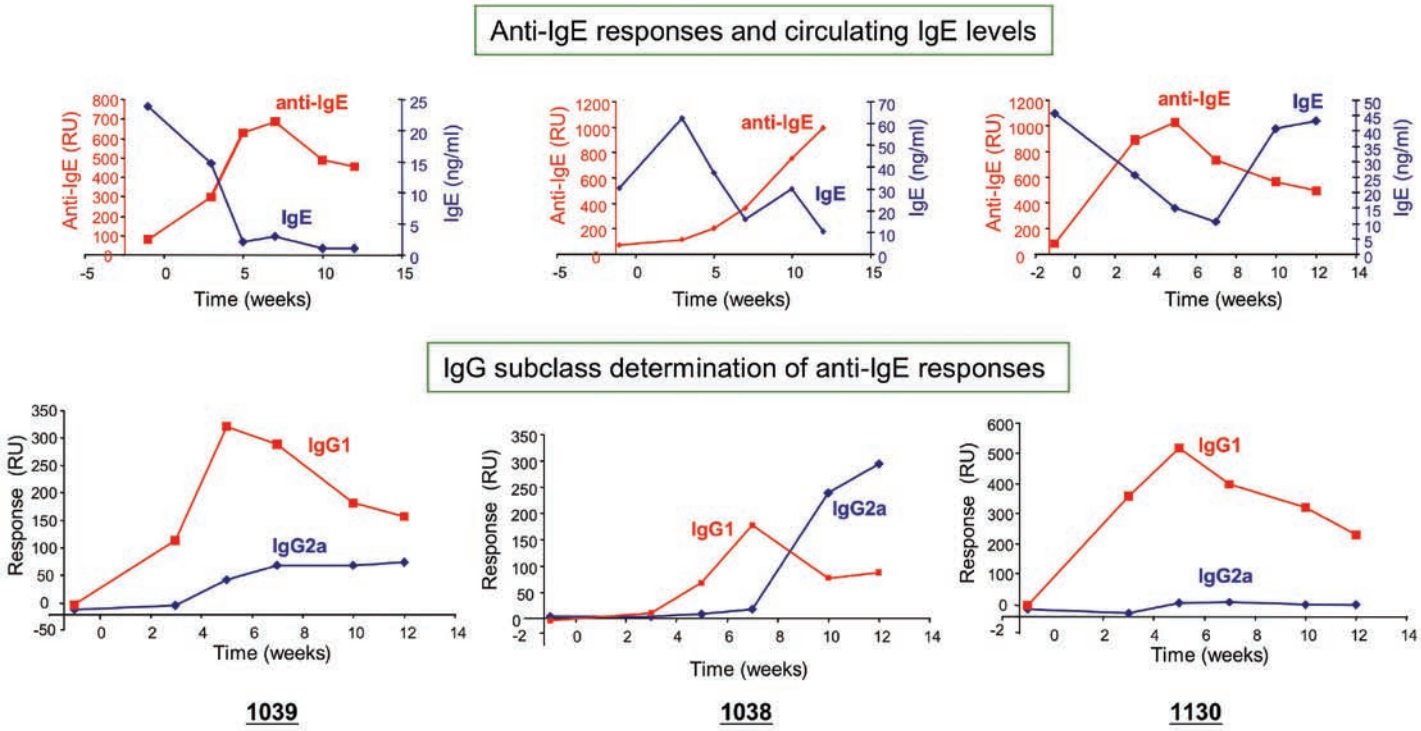

Figure 5. Comparison of total rat anti-IgE responses, circulating IgE levels and anti-IgE subclass composition in three animals. Animal 1039 (left panel) showed the most "typical" responses; 1038 (center panel) showed a continuous anti-IgE response and particularly strong late IgG2a induction; 1130 (right panel) showed only a transient IgE reduction and no induction of IgG2a.

Rat anti-IgE immune responses correlate with reduced serum IgE levels

The levels of circulating IgE in serum from treated rats were compared with the anti-IgE immune responses measured using Biacore T100. In general, there was a good inverse correlation observed between the anti-IgE responses and the levels of circulating IgE (*i.e.* the stronger the anti-IgE response, the lower the level of serum IgE). Examples of the anti-IgE/IgE correlations are shown in Figure 5.

By comparing the immune responses and IgE levels at the different time points for each individual animal, a correlation coefficient can be calculated. Calculations were performed in tandem with the Biacore T100 anti-IgE responses and the corresponding ELISAs. This shows the variation in the degree of correlation among individual animals. The degree of inverse correlation was stronger in 13 of 16 animals examined using Biacore T100 data, suggesting that this assay is quantitatively more accurate and reproducible than the ELISA.

Rapid determination of IgG subclass switching during rat immune responses

Sequential injection of isotype-specific (*e.g.* IgA, IgM) or IgG subclass-specific antibodies over the sensor surface after the serum sample provides rapid, convenient determination of the isotype and subclass composition of the responses. Little or no IgA or IgM was detected in the rat serum responses to the immunotherapeutic protein. Some differences in IgG subclass were readily apparent, however, which may be significant in relation to the general anti-IgE profiles and IgE neutralizing effects observed among the different animals.

The most typically observed combined data pattern was i) a peak of anti-IgE response at weeks 5-7, ii) a primarily IgG1 subclass composition initially (transiently peaking), with a later onset of IgG2a and iii) a corresponding decrease in IgE to below 10 ng/ml before week 7 (remaining low until at least week 12). It was interesting to note therefore, that in one animal that showed an atypical, non-peaking rise in

anti-IgE response over the twelve-week study, there was a particularly strong induction of IgG2a antibodies after week 7, which may more than fully compensate for the reduction of IgG1 antibodies in this case. In contrast, animal 1130 showed only a transient reduction in IgE levels, which rose sharply again after week 7, despite a seemingly "typical" anti-IgE response. Subclass determination, however, showed that there was no detectable late IgG2a induction, which may explain the failure of this animal to maintain reduction of circulating IgE over the 12-week period. These examples are shown in Figure 5.

Primate serum anti-IgE responses correlate closely with those obtained by ELISA

Cynomolgus monkeys were used as a primate model system. For these assays, two chimeric opossum/Cynomolgus monkey IgE C domain proteins (OCO and OCOCO) were used for immunizations, and serum responses were detected by immobilizing a recombinant IgE containing exclusively primate-derived C domains to the sensor surface (Figure 8B). OCO-treated animals were administered booster immunizations at weeks 3, 7, 29 & 31, whereas the study group treated with OCOCO was given boosters at weeks 3 & 7 and terminated at week 18. Although the peak responses varied between around 250 to 3500 RU among the eleven animals examined, the time-dependent patterns of primate anti-IgE responses were somewhat less varied than those seen in the rat study. In general, they showed a peak response at around week 9, followed by a steady decline. The OCO-treated group all showed a strong increase following the booster immunizations at weeks 29 & 31, however, reaching comparable or higher levels than the week 9 peak levels by the end of the study at week 35 (Figure 6C).

Direct comparison of results using Biacore T100 with those from Resistentia's standard ELISA method showed a generally close correlation in the general patterns observed. It was interesting, however, that in 5 of 11 animals examined the peak level and overall response profiles were shifted earlier in time in the Biacore T100 data. Similar observations have been reported previously in biopharmaceutical immunogenicity applications, and cited as an advantage of this approach[2]. This may reflect the ability of real-time Biacore assays to detect low/medium affinity antibodies

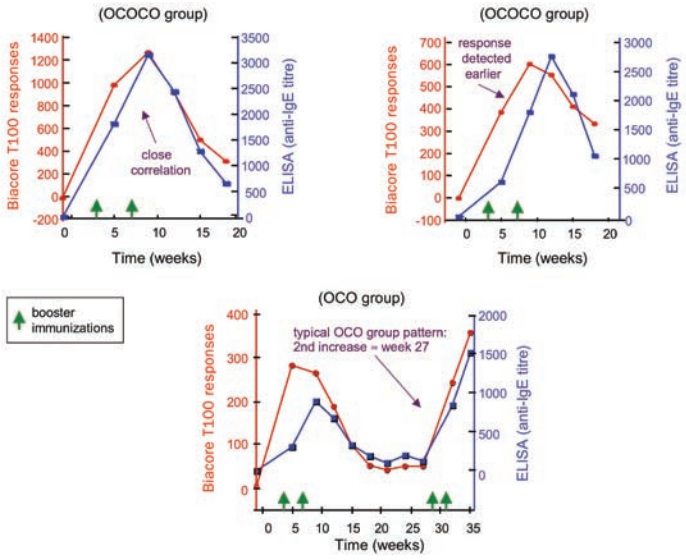

Figure 6. Comparison of primate anti-IgE serum responses using Biacore T100 and ELISA. Correlation plots for individual animals.

with rapid kinetic properties, which may well be lost during washing procedures necessary in the ELISAs. Figure 6 shows direct comparison examples for serum anti-IgE responses in three individual animals.

Superior reproducibility compared to ELISA

Since the two studies were carried out in parallel on the same primate serum samples, it was possible to directly compare the reproducibility of the methods. As shown in Table 1, the Biacore T100 assays showed a very much higher degree of reproducibility, with intra- and inter-assay CVs of 0.35% & 3.7%, respectively, compared to 22% & 38% in the equivalent ELISAs.

Table 1. Reproducibility of serum anti-IgE measurements. Measurements were carried out in triplicate in five separate assays, using identical sample sets from 8 different animals.

	Biacore T100	ELISA
Intra-assay CV	0.35%	22%
Inter-assay CV	3.7%	38%

Assessment of dosing regimes by rapid kinetic monitoring of anti-IgE antibody: antigen binding stability

It was not possible to correlate primate anti-IgE serum responses directly with free circulating IgE levels due to a lack of a suitable assay. The real-time, high information content data from Biacore T100 did, however, enable assessment of the quality of anti-IgE antibodies produced in the animals over the course of the immunization period. This was achieved by monitoring the relative stability of binding of serum antibodies to their target antigen over the course of the immunization period. Since the dissociation rate of the antibody-antigen interaction is independent of the antibody concentration in the serum samples, comparing the dissociation rates of samples taken from different time points in the study gives a good overview of stability changes in response to repeated immunizations. Similar methods have been used in human clinical immunogenicity studies related to the use of biopharmaceuticals[2].

Relative dissociation rates were calculated from the normalized anti-IgE serum response sensorgrams by taking the linearly fitted slopes of the dissociation phases, immediately after the end of sample injections, over a 2-minute period. Dissociation slopes were calculated from all available time point samples, providing analysis over weeks 5–35 for the OCO group (animals 8 to 12), and over weeks 5–18 for the OCOCO group (animals 13 to 16). As shown in Figure 7, there were clear reductions in the slopes observed for all eleven animals. The dissociation rates exhibited by the serum anti-IgE antibodies therefore became increasingly slow over the course of the immunization period, indicating an increase in antibody binding stability.

Estimates from these data indicated that antibody binding was relatively stable by week 5, with a $t_{1/2}$ range of approximately 13–40 minutes. Despite this, the pattern of responses observed demonstrated a further improvement in binding stability over time, suggesting affinity maturation was occuring. In the OCO group that was studied over a longer period (Figure 7A), it was apparent that the main effects of this kinetic maturation of the anti-IgE response occurred during the period between weeks 5 to 20, *i.e.*, before the third booster immunization at week 29. The strong increases in anti-IgE response seen in the OCO group animals after the final two booster immunizations appear to reflect, therefore, an increase in the magnitude of an antibody response that has already matured in terms of its kinetic properties.

This type of rapid kinetic analysis may, therefore, be very valuable in the optimization of dosing regimes for immunization programs.

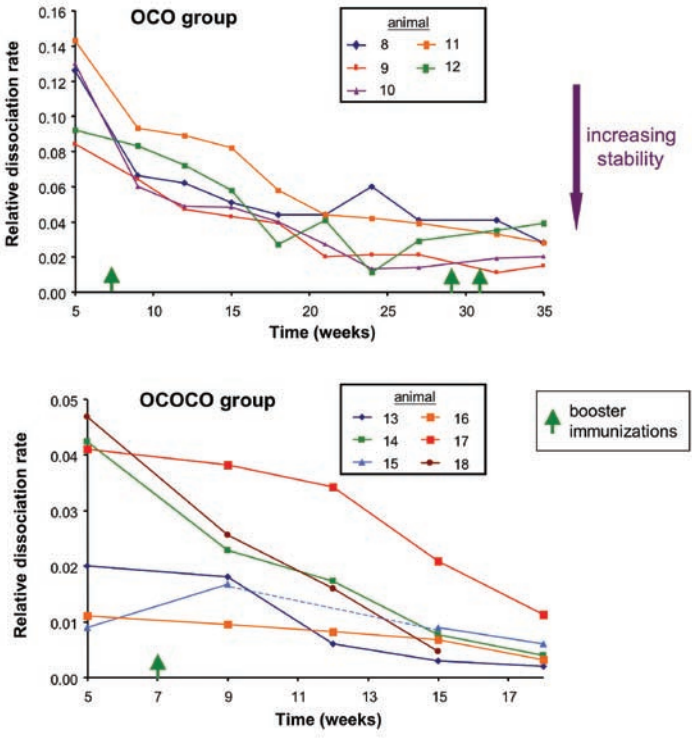

Figure 7. Changes in binding stability of primate serum anti-IgE responses during the immunization study. Slopes taken from the early dissociation phases of normalized sensorgrams corresponding to each time point are plotted against time. Note that these plots begin at week 5, after the first booster immunization (week 3). The broken section of the line for Primate 15 indicates one missing data point for week 12.

Summary

- The recombinant IgE-derived immunotherapeutic proteins developed by Resistentia Pharmaceuticals AB show excellent immunogenic properties in the two animal systems tested, providing the basis for an effective immunotherapeutic approach to the treatment of allergy and asthma.
- Biacore T100 provided high information content characterization of anti-IgE responses over time and in relation to immunization dosing and adjuvance regimes
  - Increased anti-IgE responses correlated strongly with reductions in circulating IgE levels, demonstrating neutralizing activity of the immunotherapeutic protein -induced antibodies
  - Subclass analysis showed a clear correlation between subclass switching patterns and effects on circulating IgE levels among different individuals
  - Rapid kinetic profiling of primate anti-IgE responses provided information on the quality of the antibody response over time and in relation to the dosing regime.
- Response data showed a good general agreement with ELISA, but showed a much higher level of reproducibility.

Methods

IgE and anti-IgE ELISAs were carried out using standard methodologies at Resistentia Pharmaceuticals AB. Biacore T100 assays were performed using Series S Sensor Chip CM5 and an assay temperature of 37°C. Standard amine coupling procedures were used to immobilize a commercial rat IgE protein (rat study) or a Cynomolgus monkey IgE-derived recombinant protein (primate study) onto the sensor surface. An overview of the assay setups for the two animal studies is shown in Figure 8.

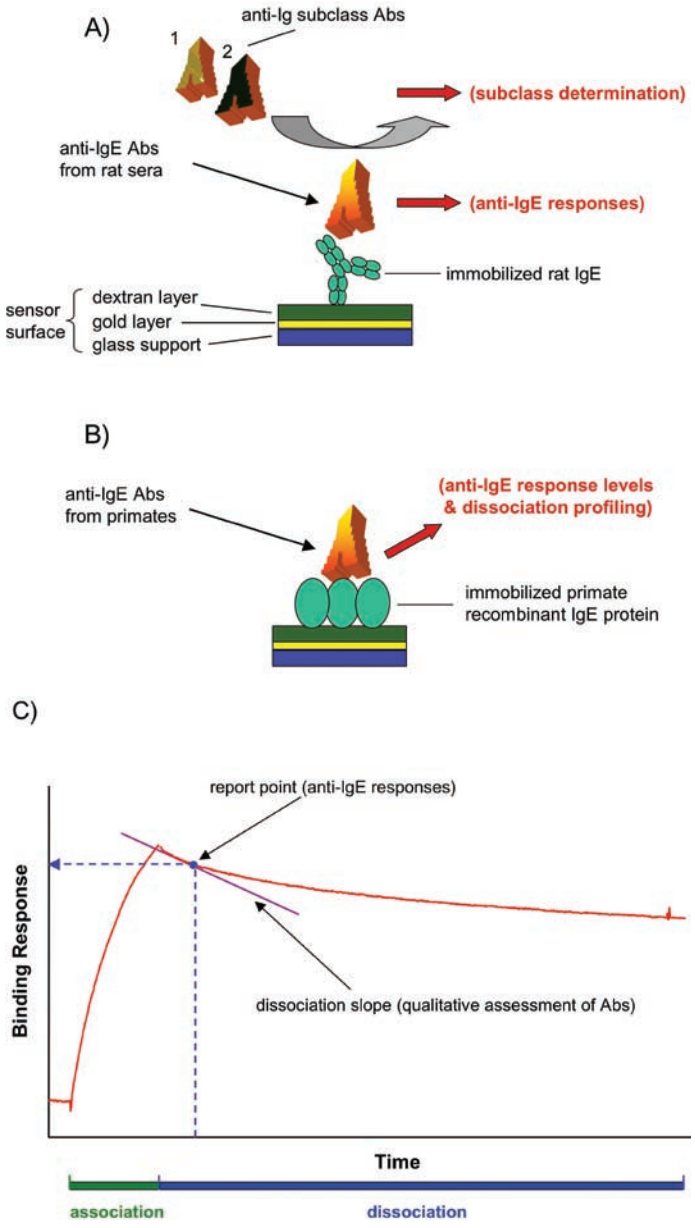

Figure 8. Overview of assay setups used in the immune response assays. A) Rat study: commercial rat IgE protein immobilized to detect anti-IgE Abs in immunized animals. Further serial injections of IgG subclass-specific Abs s identified subclass of antigen-bound serum anti-IgE Abs. B) Primate study: recombinant Cynomolgus monkey IgE immobilized on the sensor surface. C) Derivation of data from sensorgrams: Binding of proteins on the sensor surface generates an SPR signal that is monitored in real time over the whole binding interaction (total interaction time 30 minutes in the example shown). Anti-IgE and subclass responses were determined from single report points, taken from specific time-points on the sensorgrams. Qualitative assessment of Abs was based on slopes taken from the early dissociation phase. In practice, the response level and dissociation profiling data were obtained from separate experiments, with sensor surface preparation and other conditions tailored for the specific assay.

References

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