The regulation of adaptive and innate immune responses: biomolecular interactions at cell surfaces

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Reports on kinetic analyses of biomolecular interactions central to the cellular response of both the adaptive and innate immune systems are reviewed here. Although the studies are wide in scope, they share the hypothesis that the kinetic profiles of interactions between receptors and their ligands may regulate cellular function, for example, the response of a T-cell to an antigen: MHC complex or the response of natural killer (NK) cells to virally infected cells. Label-free interaction analysis with Biacore™ systems provides a rapid and reliable means to obtain accurate kinetic data on interactions between molecules of the immune system, which may be of importance in the search for effective therapeutic reagents targeted at these interactions.

T-cell receptor recognition of peptide antigen: MHC complexes

The ability of higher organisms to distinguish between foreign and self antigens is perhaps the most exquisite example of regulation in biology. B-cell and T-cell receptors not only recognize and engage foreign antigens but are also able to recall the molecular signature of the antigen and trigger the immune system to mount a rapid and effective response to an assault by the same antigen years or even decades after the initial encounter.

The group of Mark M. Davis at Stanford University School of Medicine has speculated on how the remarkable specificity of T-cell antigen recognition may be regulated (1). A Biacore system was used to analyze the contribution of individual amino acid residues in both an antigenic peptide and its class II MHC presenting partner. The association rate constant (k_a) and affinity dissociation constant (K_d) for interactions between the T-cell receptor (TCR) and combinations of MHC/antigen peptide complexes were measured.

These values were then incorporated into an algorithm yielding an estimate of the activation energy of association for a given interaction. By using point-mutated analytes, a ϕ value (a measure of the contribution of a given amino acid to the activation energy of association in relation to its contribution to the free energy of binding), was attributed to individual amino acid residues and the contribution of each residue to the formation of a transition complex was estimated.

Single point mutations were generated in regions of either the antigen peptide (moth cytochrome c) or the α chain of an associated class II MHC molecule which, according to crystal structure data on other complexes, interact with the TCR. The complexes were immobilized on a sensor surface and soluble TCR was then injected over the surface. All measurements were standardized by comparison with a wildtype complex immobilized in a parallel flow cell.

Strikingly, residue changes on the antigen peptide affected the dissociation rate but not the association rate. In contrast, MHC contacts with the TCR were largely responsible for the association rate while having much less influence on dissociation and hence the overall stability of the complex. This has led to the hypothesis that the MHC component enables the TCR to screen potential complexes while the antigen acts to stabilize the interaction (Fig 1). This model is consistent with data indicating that most peptides lie partially concealed within a deep cleft in MHC molecules and indicates that peptides must be actively presented to the TCR in a particular orientation in order to form a stable complex (2).

On the strength of kinetic data derived from the mutational analysis experiments using a Biacore system, a dual binding mechanism for antigen engagement by the TCR is proposed. In this model, rigid CDR1 and CDR2 domains scan the MHC component of the presenting complex. Upon presentation to the TCR, the more flexible CDR3 domain is then able to fold over the antigen component in a stable, induced fit. This type of mechanism could explain why TCRs are inherently cross-reactive, because the second stage folding into the peptide binding region could assume a very large number of conformations.

Recognition of IgG by low affinity Fcγ receptors

Immune complexes comtprised of autoantibodies and antigen can initiate inflammatory responses by binding to immunoglobulin (Ig) Fc receptors (FcRs) on hematopoietic cells. The complexes contribute to tissue injury in a number of autoimmune diseases including rheumatoid arthritis.

FcγRIII is a low affinity receptor for IgG, with particular binding preference for IgG_1 in complex with antigen. FcγRIII binds to IgG at an epitope in the lower hinge region of both the heavy and

light chains. A conserved oligosaccharide attached to asparagine residue 297 (Asn^{297}) occupies a unique cleft very close to the FcγRIII recognition motif (Fig 2) and may contribute to receptor binding by stabilizing the hinge region and maintaining the tertiary structure of the molecule. Sergei Radaev and Peter Sun at the National Institutes of Health in Rockville tested this hypothesis by using Biacore assays to compare native glycosylated whole IgG or Fc fragments and enzymatically deglycosylated counterparts in interactions with FcγRIII (3). They also investigated the potential of peptides derived from the lower hinge region as therapeutic antagonists designed to inhibit the interaction between IgG and FcγRIII. To determine the role of carbohydrates in receptor recognition, FcγRIII was immobilized on a sensor surface and exposed to different concentrations of native IgG_1, deglycosylated IgG_1 or Fc fragments. Binding of Fc fragments to FcγRIII was effectively abolished while the affinity of deglycosylated IgG_1 for the receptor was reduced 10-fold compared to native IgG_1.

In order to propagate signals leading to an inflammatory response by target cells, the receptors must cluster on the cell surface. Small peptides that can mask the binding site for IgG_1 but fail to cluster FcγRIII might therefore function as antagonists. Peptides based on the lower hinge region of Fc were synthesized and immobilized on a sensor surface. Various concentrations of FcγRIII were then injected over the prepared surfaces and binding affinities were measured. Although many interacting peptides were identified, the affinity of even the strongest binders was nevertheless some 20-fold weaker than native Fc.

Using a competition assay format in which Fc-derived peptides were immobilized on a sensor surface and exposed to FcγRIII in the presence of different concentrations of Fc fragments, binding of FcγRIII was progressively blocked as the Fc fragment concentration increased. This suggests that the peptides and the native Fc fragments share the same epitope on FcγRIII.

Biacore assays were thus employed in a variety of formats in this study to yield quantitative data suggesting that the oligosaccharide component on Asn^{297} of IgG_1 is important for the interaction of IgG with its low affinity receptor and that peptides derived from the lower hinge region of IgG may be useful as antagonists in the treatment of diseases with autoimmune IgG-mediated etiology.

Cell surface receptors of the innate immune system

Phagocytes and natural killer (NK) cells present a first line of defense against pathogens. Although less refined in its mechanisms than the targeted B-cell and T-cell responses of adaptive immunity, the innate defense system is nevertheless an indispensable barrier against invasive organisms. One important role of the various forms of phagocytes of the blood and tissues is to internalize and degrade foreign particles including bacteria while NK cells destroy host cells transformed or infected with viruses.

The delay by T-cells in consolidating an effective response to a viral assault gives NK cells a vital defensive role in controlling

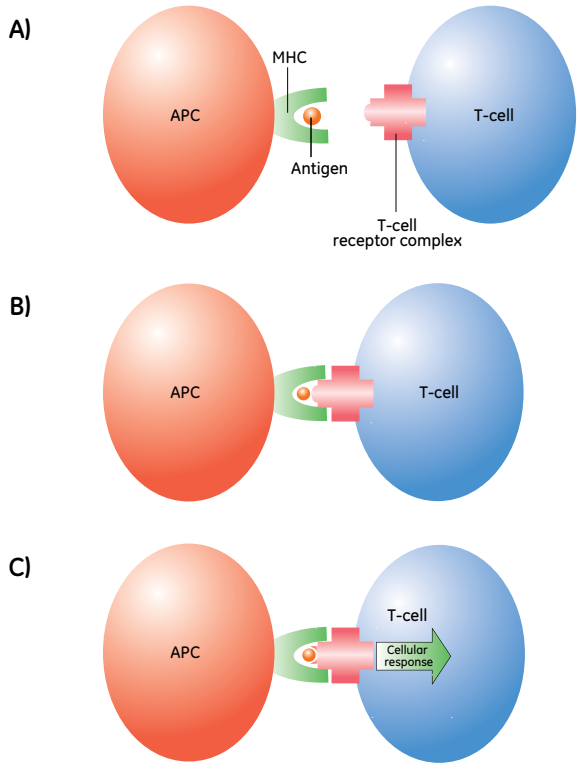


Fig 1. A) A T-cell approaches an antigen presenting cell (APC) carrying an antigen displayed in association with a major histocompatibility complex (MHC); B) The TCR binds to the MHC molecule in a low affinity interaction; C) Recognition of self-MHC induces a conformational change in the TCR, enabling an induced fit, high affinity interaction with antigen.

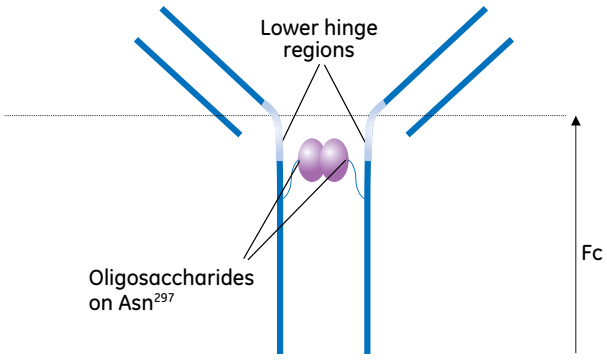


Fig 2. The relative position of carbohydrates carried on Asn^{297} of both heavy chains of IgG and occupying the central cleft of the molecule. The lower hinge regions (highlighted) contains the recognition sequences for the low affinity IgG receptor, FcγRIII.

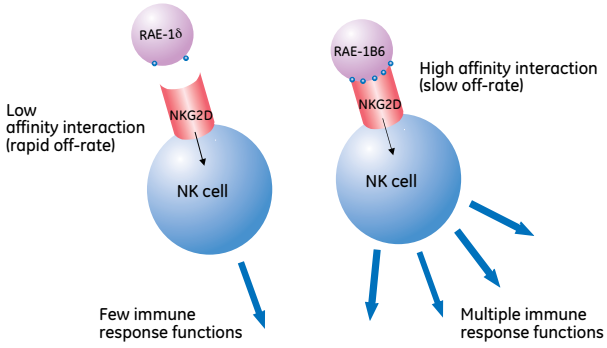


Fig 3. The off-rate of ligands may differentiate between NK cell responses that are highly sensitive to receptor stimulation and those that require prolonged stimulation. The reasons for the great difference in affinity between RAE-1δ and RAE-1B6 despite their structural similarity may be probed by restricted point mutagenesis studies based on the few disparate sites in their binding sites for NKG2D.

the infection by killing infected target cells and thus inhibiting viral propagation. NK cells are activated through cell surface lectin-like receptors. Leonidas Carayannopoulos and colleagues at Washington University School of Medicine used a Biacore system to study the mode of interaction between the receptor, NKG2D and MHC class I-like ligands constitutively expressed on certain tumors (4).

Recombinant NKG2D was expressed in insect cells and purified. The protein was immobilized via neutravidin on a sensor surface and exposed to the known ligands, RAE-1δ, H60, and RAE-1B6. Although H60 and RAE-1B6 have only approximately 20% sequence identity, they both bound to NKG2D with a similar affinity (K_D of 20 to 30 nM). On the other hand, the affinity of RAE-1δ was some 30-fold lower despite its structural similarity to RAE-1B6. By fitting the binding data to a biexponential model, this weaker affinity appeared to be a consequence of both slower association and faster dissociation. Among the suggested mechanisms behind this apparent two-step binding process are concentration-driven aggregation of RAE-1δ and recognition by NKG2D of two asymmetric binding sites on RAE-1δ. This latter model has a precedent in the mode of binding between NKG2D and another ligand termed MICA.

The difference in affinity between RAE-1B6 and RAE-1δ for NKG2D, despite high sequence identity, may indicate those residues that should be targeted in mutation analyses in order to probe any apparent functional redundancy in this dual ligand system. It is possible, however, that the function of NKG2D may be differentially regulated by the characteristic kinetic profiles of alternative ligands. For example, the rapid dissociation rate may qualify RAE-1δ as a rather discriminatory regulator of NKG2D functions, triggering only the most sensitive responses while the more slowly dissociating RAE-1B6 may promote a much broader range of functions (Fig 3). The functional importance of this type of duality is suggested by the fact that mice lacking H60 nevertheless express RAE-1B6, a protein that although structurally dissimilar to H60, interacts with NKG2D with similar kinetics.

The kinetic data in this report were obtained entirely from Biacore assays, enabling the authors to propose that kinetic behavior may be a regulatory parameter in itself, differentiating between signaling pathways via the activation of a common receptor by different ligands. It is possible that multiple specificity of receptors of the innate immune system may enable a limited repertoire of NK cell clones to survey a wide array of pathogens.

Characterization of the interaction of natural and foreign ligands with complement receptor 2

Complement receptor 2 (CR2) is a transmembrane glycoprotein expressed on B-cells. Binding of CR2 with the cleavage products of C3 (iC3b or the subsequent serum protease-derived product, C3d) generated during complement activation is involved in a range of responses e.g. generation of immunological memory, Ig class switching and tolerance. Maria Rosa Sarrias and colleagues at the University of Pennsylvania in Philadelphia used label-free interaction analysis to differentiate the kinetics of interactions between C3-derived complement components and CR2 (5). Additionally, the interaction between Epstein Barr virus (EBV) antigen gp350 (which has been shown to elicit responses similar to "natural" CR2 ligands in B-cells) and CR2 was characterized.

CR2 or C3-derived components were firstly biotinylated and immobilized on a sensor surface preconditioned with streptavidin (Sensor Chip SA). Biotinylation of the immobilized proteins was carefully controlled in order to mimic orientation at the cell surface. CR2 was cloned and expressed as a fusion protein with a C-terminal subunit of "biotin carboxyl carrier protein" (BCCP), an active subunit of acetyl-CoA carboxylase from *E. coli*. This subunit contains a lysine residue that acts as a biotin acceptor and thus directs biotinylation to that site. This strategy enabled CR2 to be biotinylated on its C-terminal BCCP tag. C3 fragments were biotinylated on the thiol group of Cys^{988}, an amino acid residue that participates in the formation of an intramolecular thioester bond. Following cleavage using methylamine and biotinylation at this site, C3 was then cleaved into its degradation fragments, iC3b and C3d (Fig 4).

A truncated fragment of the EBV surface glycoprotein, gp350, bound to CR2 and the binding data closely fitted a 1:1 model. The calculated K_D of 45 nM is some 4-fold lower than that determined when whole gp350 bound to CR2 in a cell-based assay (6), a difference that could indicate that other regions of gp350 influence the interaction.

Kinetic analysis using Biacore assays suggested that interaction of C3 cleavage fragments with CR2 was more complex, fitting a bivalent analyte binding model, which is consistent with data from other studies. Indeed, in addition to the C3d site found by X-ray crystallography to be involved in CR2 binding (7), a peptide covering residues 1199–1210 of C3 bound to immobilized CR2 in Biacore assays. This peptide, which was unable to inhibit binding of C3d to CR2, was characterized by fast dissociation and suggests the presence of a further discrete CR2 binding site on C3d.

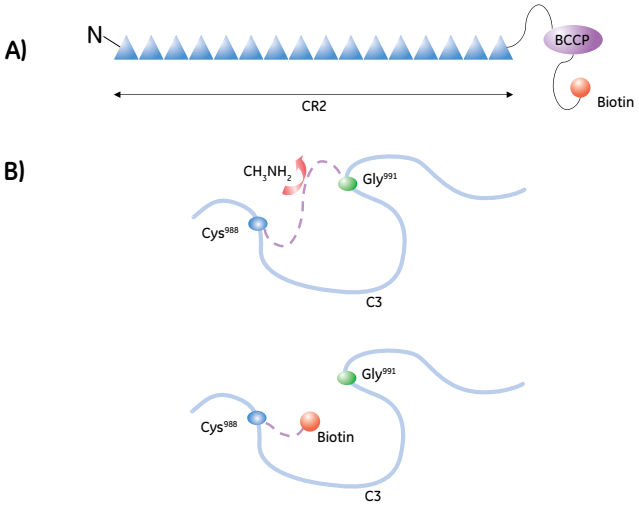
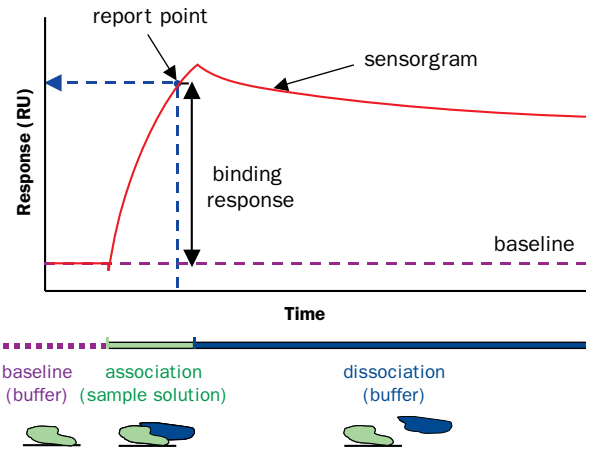


Fig 4. A) CR2 was biotinylated at its C-terminus by firstly producing the protein as a construct with the BCCP fragment of *E. coli*-derived acetyl-CoA carboxylase that functions to direct biotinylation at the ligation site on exposure to the enzyme, BirA; B) Biotinylation of C3 on Cys^{988}. The intrachain thioester bond between residues Cys^{988} and Gly^{991} was cleaved using methylamine. iC3b and C3d fragments were generated from the same treated stock of biotinylated C3.

Monitoring protein interactions with Biacore systems

Biacore systems monitor protein interactions in real-time using a label-free detection method. One of the interacting molecules is immobilized onto a sensor surface, while the other is injected in solution and flows over the sensor surface. As molecules from the injected sample bind to the immobilized molecules, this results in an alteration in refractive index at the sensor surface that is proportional to the change in mass concentration. Using the phenomenon of surface plasmon resonance (SPR), these changes are detected in real-time and data are presented as a sensorgram (SPR response plotted against time). Sensorgrams display the formation and dissociation of complexes over the entire course of an interaction, with the kinetics (association and dissociation rates) revealed by the shape of the binding curve.



The sensorgram provides real-time information about the entire interaction, with binding responses measured in resonance units (RU). Binding responses at specific times during the interaction can also be selected as report points. More information can be found at **www.gelifesciences.com/biacore**.

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Acknowledgements

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