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Cross-linking of Fc gamma receptors and surface antibodies: theory and application. FREE

N R Sinclair

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LETTER TO THE EDITOR

Sir:

The article by C. Wofsy and B. Goldstein entitled "Cross-linking of Fcγ Receptors and Surface Antibodies: Theory and Application" raises the question of whether the co-cross-linking of antigen-receptors with Fc receptors simply reduces the positive signal induced by the cross-linking of antigen-receptors (passive inhibition) or actually provides a negative signal of its own (active inhibition). The simulation models developed and their correspondence with actual data seem to favor active inhibition, but, as correctly stated by Wofsy and Goldstein, these do not rule out passive inhibition. However, the future experiments they suggest seem to come no closer to distinguishing between passive and active inhibition. May I suggest an approach that will do so.

The distinction must be based on developing experimental conditions in which the positive signal through the antigen-receptor is no longer required for the response being measured. The most obvious way to do this is to allow the positive signal sufficient time to take place and then study a response sufficiently soon afterwards so that any reduction in positive signaling will be without effect. Under these conditions, removal of positive signals will not suppress, while delivery of a negative signal may be inhibitory.

This is the experimental approach that we took when we first proposed a model for inactivation of lymphocytes based on the cross-linking of antigen-receptors with Fc receptors through a bridge composed of antigen and antibody to the antigen (1). Mice were injected with antigen on Day 0, and then given varying quantities of antibody to antigen on Day 1. We predicted that there would be an amount of antibody which would give maximal inhibition (inducing optimal cross-linking of antigen-receptors with Fc receptors), while greater quantities of antibody would simply displace antigen from the antigen-receptors. We looked at an early response which was likely not to be inhibited by the removal of antigen. This is precisely what we found and reported. This result told us that massive amounts of passively administered antibody to antigen will not suppress an immune response that does not require antigen any more, but a smaller amount of antibody would do so. Similar to the reasoning by Wofsy and Goldstein, we assumed that co-cross-linking would depend on the correct balance of antigen and antibody to antigen. Our experimental results (1) could only be explained on the basis of active inhibition, that is, the delivery of a negative signal following the co-cross-linking of the antigen-receptor with the Fc receptor on cells which were already stimulated to give the response measured.

Similarly, in the case of antibody to the antigen-receptor, the most convincing experiment will be to activate B cells with $F(ab')_2$ anti-receptor antibody, attempt inactivation later with varying amounts of intact IgG anti-receptor antibody, and then to study a B cell activation step, such as a proliferative response, soon enough so that the loss of stimulatory signals from the $F(ab')_2$ antibody will not matter, but at a time far enough in the future for the negative signal, due to co-cross-linking, to have an effect. Maximum suppression, due to optimum co-cross-linking of antigen-receptors with Fc-receptors, should then be easily demonstrated. This would be the first confirmation of our 20 years of observations.

Lastly, may I point out that whereas the effects of anti-receptor antibodies are good model systems to study, what they are modeling is suppression of immune responses by end-product antibody *to antigen*. Antibody with this specificity is likely to be the most frequent cause of co-cross-linking; the regulated cell produces this regulator to bind to antigen which these cells must also have, if they are to be stimulated. On the other hand, *anti-receptor* antibodies must be formed by cells other than the ones being regulated (2). Many immunologists, studying the inhibitory effects of anti-receptor antibodies with various specificities, seem to have lost sight of these considerations: all, in fact, except O'Garra and coworkers (3).

Nicholas R. StC. Sinclair
The University of Western Ontario
Dept. of Microbiology and Immunology
Health Sciences Centre
London, Canada N6A 5C1

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In reply:

Thank you for your letter of October 10, 1990, offering us the opportunity to comment on Dr. N. Sinclair's interesting letter.

There is a spectrum of B cell responses to antigen, occurring on very different time scales (seconds to days). Most responses appear to be inhibited by the co-cross-linking of cell surface immunoglobulin (sIg) and Fcγ receptors by anti-sIg IgG or antigen-IgG complexes, although not all types of Fcγ receptors may participate in inhibition (1). For each response, the question of whether inhibition is active or passive arises.

The experiments of Drs. Sinclair and Chan (2) are particularly suited to the long time response they studied: the production of antibody-secreting cells, measured five days after murine B cell activation by antigen (sheep erythrocytes). They showed that injection of antigen-specific IgG even 24 h after activation by antigen sup-

presses the production of plaque-forming cells. The inhibition cannot be explained by reduction of the activation signal (sIg cross-linking). At the highest concentration ratio of injected IgG to antigen, the production of antibody-secreting cells shows no inhibition. Sinclair and Chan argue convincingly that at these IgG concentrations all antigenic sites are blocked and neither cross-linking nor co-cross-linking occurs. At intermediate concentrations of injected IgG, where antigen-IgG complexes are more likely to have some free antigenic sites, making cross-linking and co-cross-linking possible, inhibition is observed. The full concentration-dependent response profile is consistent with the view that inhibition of this response occurs by an active mechanism initiated by co-cross-linking.

Dr. Sinclair suggests that similar experiments be done with anti-sIg IgG and its $F(ab')_2$ fragments. Phillips and Parker (3) measured the production of antibody-secreting cells as a function of the time between $F(ab')_2$-induced B cell activation and the addition of intact IgG. They found that if activated cells were exposed to IgG up to 48 h after stimulation, the response was inhibited. However, the IgG concentration dependence of inhibition was not reported.

The approach Dr. Sinclair outlines in his letter is useful for detecting certain types of active inhibition, but clearly not all types. For example Rigley et al. (4) have observed that cross-linking of sIg on B cells causes the rapid breakdown of phosphatidylinositol-4,5-bisphosphate, a response that involves a G protein that couples sIg to phospholipase C. They propose that co-cross-linking of sIg and Fcγ receptors inhibits this response by uncoupling the G protein from the sIg. This form of active inhibition requires the presence of co-cross-linking during activation. Indeed, negative signals that are the result of some form of competitive inhibition must be present along with the activation signal in order to be effective.

Carla Wofsy
Department of Mathematics and Statistics
University of New Mexico
Albuquerque, NM 87131

Byron Goldstein
Theoretical Biology and Biophysics Group
Los Alamos National Laboratory
Los Alamos, NM 87545

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