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A matter of debate

How immunologists describe immune responses can profoundly influence the types of experimental questions addressed in future studies.

When do immune cells respond and when do they remain quiescent? How can friendly signals be distinguished from those that might be harmful? Can the lack of 'conventional' hallmarks of immune cell activation be called a null response or does it instead represent some form of immunologic tolerance? Can characteristics of immune responses occurring in sterile splenic environments be accurately extrapolated to describe immune responses happening at other sites in the body?

Those questions and more were discussed during the town-hall debate "Friendly or Dangerous Signals" at the 2006 Keystone Conference on Tolerance, Autoimmunity and Immune Regulation held in Breckenridge, Colorado. The idea for a debate on the nature of immune responses was conceived by Matthias von Herrath (La Jolla Institute for Allergy and Immunology), who also served as the moderator. Panelists included Neil Greenspan (Case Western Reserve University), Eyal Raz (University of California San Diego), Polly Matzinger (National Institutes of Health) and Rolf Zinkernagel (Institute for Experimental Immunology). What came across to the audience as a belief shared by all the panelists was that immune responses are complex.

In this issue of *Nature Immunology*, each of the panelists recapitulates their viewpoint for a wider immunology audience. Some points of agreement can be found, although ongoing disagreements on other issues still engender debate. The panelists expressed agreement about the idea that often the parameters used to characterize immune responses or to conclude that a immune response was or was not elicited might not be the most appropriate or relevant to describe the biological phenomena being studied. Overinterpretation of experimental data and extrapolation of data sets to other settings (that might not share similar environmental cues or cellular participants) were also cited as factors that lead researchers to draw conclusions that might not accurately reflect biological responses to pathogenic or commensal organisms. Investigators simply might not be using the most relevant assay, or combination of assays to measure multiple parameters simultaneously, to accurately describe immune responses.

That idea might be shocking to many experimentalists who study immunology, and it might reflect a conditioned or dogmatic approach toward experimental design. Often, to 'dissect' a given biological issue, scientists attempt to reduce the number of variables in the sampling process. How many have heard the admonition 'vary only one parameter at a time'? Indeed, some conditions used for *in vitro* experiments are skewed to deliver the maximal response, such as assays measuring proliferation or cytokine release. Maximizing such responses might be essential for achieving statistical significance. Any number of commercially available kits can be purchased that have been optimized such that reproducible results can be obtained in diverse laboratories by many hands or by the same hands on different days. Also, such standardized assays are easier to do and allow data to be amassed more quickly. Such reproducibility is taken as validation of the results, and perhaps the interpretation, of such experiments. But if an irrelevant parameter is measured, even if statistical differences can be detected in specific experimental conditions, does that information actually increase understanding of a physiological response?

Panelists also agreed that an inherent bias exists in how experimental results are interpreted. All too often an experiment attempts to frame a question as an 'either-or' choice. The T helper type 1 (T_H1)–T_H2 polarization of effector $CD4^+$ T cell function is such an example. After stimulation, production of interferon-γ indicates a T_H1 response and production of interleukin 4 indicates a T_H2 response, whereas if neither cytokine has been produced the response is described as 'null'. But is nothing really happening? Many reports have contributed to the knowledge of how 'signature' responses arise and how polarity is maintained *in vitro*, yet less well known are the physiological signals that trigger appropriate responses *in vivo*. As seen with the identification of interleukin 17–producing $CD4^+$ T cells, attempting to qualify responses in 'either-or' terms might lead researchers to miss more relevant immune events. Editors and referees are not absolved from contributing to such biases.

In some cases, cells stimulated *ex vivo* are placed back into an animal, typically a mouse, to demonstrate physiological relevance. In those situations, the recipient mouse is essentially treated as a test tube. Although such experiments provide information about what responses can occur, whether such responses actually do occur in a physiologically relevant scenario and in a time frame relevant to a person challenged with a pathogen is not at all certain. It is likely that most, if not all, physiological immune reactions are the result of many inputs that determine the final response. Some of those influencing parameters might be subtle and perhaps are responsible for the tissue-specific characteristics of immune responses. Others, such as antigen dose and concentration and the frequency of responding cells, might have a more overt function in directing the type of immune response.

We hope this series of commentaries representing individual views on what constitutes an immune response provokes much discussion. We believe that debating such issues in public forums is beneficial to the research community. Such critical thinking should prompt reexamination of how well the experiments used to understand an immune response mimic the physiologic scenario in which the response occurs. We invite readers to comment on this series of commentaries and welcome your suggestions for other topics for which open 'debate' would also benefit the community of immunologists.