Friendly and dangerous signals: is the tissue in control?

Polly Matzinger

In their own defense, tissues send a panoply of signals that initiate immunity and guide the choice of effector class. T_H1-T_H2 and T_{reg} is far too simple a representation of the breathtaking variety of the resulting responses.

This debate, though slated to be about dangerous and friendly signals, ranged over a number of topics, including the use of words, the use of assays and the complexity of the immune system. Here I will write primarily about the signals I believe to be in charge of immunity, incorporating as best as I can some of those other topics. In some cases what I write below will resonate with what the other authors said. In other cases, we disagree. Because these commentaries are not structured to mimic the debate that occurred, it would be unwieldy to point out each instance of agreement and disagreement, so I leave it to the reader to discern these.

To respond or not?

When faced with a potential threat, the immune system has two main questions to answer. The first, 'shall I respond?', is what most models of immunology deal with. The old 'self–non-self' model assumed that the answer was 'yes' if the potential threat were foreign (as seen by the antigen-specific receptors of T and B cells). The newer pattern-recognition receptor (PRR) model[1] assumes that the answer is 'yes' if the potential threat is *very* foreign — for example, bacterial or viral pathogen-associated molecular patterns (PAMPs) as seen by the Toll-like receptors (TLRs) of antigen-presenting cells (APCs) — and the 'danger' model[2,3] assumes that the answer is 'yes' if the potential threat does damage that elicits APC-activating alarm signals from the damaged tissues.

Polly Matzinger is with The Ghost Lab, Laboratory of Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.
e-mail: pcm@helix.nih.gov

Because some of the endogenous alarm signals seem to use the same TLRs as the bacterial and viral PAMPs, many immunologists dismiss these, saying that any observed activity is really due to undetectably low amounts of contaminating endotoxin. This is not a debate that I want to get into right now. Both *in vivo* and *in vitro* examples exist that are extremely difficult to dismiss with such arguments, and for the others, time will tell. However, the argument may be a needless one, as there is a way to reconcile the PRR and danger models with one simple assumption, namely that both the PAMPs and the endogenous alarm signals belong to a common set of signals that are nearly as ancient as life itself.

Briefly, the idea is that very early in the origins of life, cells (both bacterial and eukaryotic) needed to be able to detect damage and other nonphysiological processes. This would be true both for individual cells (to detect membrane damage, heat shock and so on) and for colony-forming organisms (who might like to know that their colony-mates are sick, so that they could change their metabolic pathways, or sporulate or leave the vicinity). There are two main types of signals that could have been used very early in evolution to signal damage. The first is hydrophobicity[4]. Because life evolved in water, the hydrophobic portions ('Hyppos') of molecules are both useful and dangerous: useful because hydrophobic interactions hold membranes together, mold the shape of complex proteins and so on; dangerous because exposed Hyppos aggregate nonspecifically to form nasty aggregates. Hyppos are thus normally hidden, and they are kept hidden by a plethora of mechanisms (chaperones, lipid binding proteins and others). A suddenly exposed Hyppo, therefore, is a sure sign of injury, damage or other accident of physiology, and receptors for such Hyppos were likely to have evolved very early in life. A close look at the molecules that bind surface TLRs shows that most, if not all, of them are hydrophobic or have important hydrophobic sites, whether they originate from bacteria (for example, the immunostimulatory lipid A portion of lipopolysaccharide (LPS), which is not normally exposed on healthy bacteria) or from injured cells (for example, spatzle or the binding sites of heat-shock proteins) or from less defined sources, such as the mineral oil of incomplete Freund's adjuvant.

A second universal aspect of life (at least on this planet) is nucleic acid, and both DNA and RNA can serve as activators of APCs. Again, though it has been suggested that only bacterial DNA has sufficient quantities of unmethylated CpG sequences to serve as ligands for the internal TLRs, eukaryotic DNA is also rife with unmethylated CpG in the promoters of genes. The important thing here is that, like the lipid A portions of LPS, which are hidden in the membrane, and the Hyppos of hyaluron, which are hidden in the polymer, neither bacterial nor eukaryotic DNA is normally exposed on healthy living cells. All of these molecules can therefore serve as signs of damage and death. Putting these together, Seung Seong and I suggested that there is a category of damage-associated molecular patterns (DAMPs) that encompasses both PAMPs and alarm signals[4]. DAMPS would be useful as quorum-sensing signals to bacteria, to early eukaryotes (to initiate repair mechanisms) and to the evolutionarily more recent vertebrates (for the initiation of repair and of immunity). If we look at PAMPs (and MAMPs or microbe associated molecular patterns) and alarm signals as

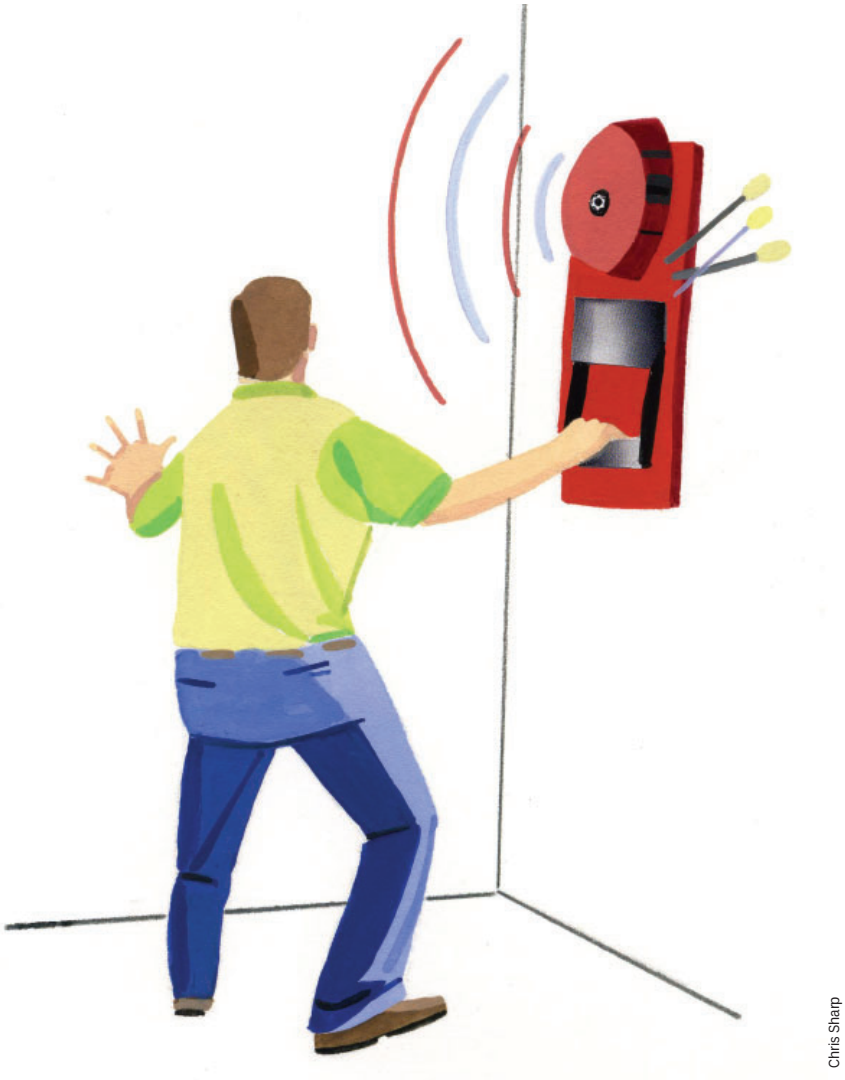
different forms of DAMPs, it is no longer necessary to argue whether these are pathogen specific or endogenous. They are both. And many of them serve to initiate both repair and immunity.

What kind of response should be made?

When an immune response is initiated, what or who decides whether to produce immuoglobulin G1 (IgG1) or IgG2a, IgG2b or IgG2c, or IgG3, or IgE or IgA? Who determines whether to activate natural killer (NK) cells or eosinophils, or superoxide-producing macrophages or cytotoxic T lymphocytes (CTLs)? Neither the old self–non-self model nor the newer PRR model (nor the original version of the danger model) incorporated this question and we are very far from getting an answer. However, after 13 years of looking at immunity through the assumption that responses are initiated by alarm signals from injured tissues, I have begun to think that the tissues may have more control over immunity than we have previously thought. Perhaps they also send signals that influence the effector class.

Most of us were taught that the choice of effector class is based on the pathogen we are facing (for example, we make IgE to worms and CTLs to viruses). I am no longer so sure of this, but have begun to consider the possibility that the ultimate control lies with the tissues in which the response occurs, rather than with the pathogen against which it is directed. Eyal Raz (in this debate and in the literature) has pointed out that the lung influences its macrophages in ways calculated to preserve lung function as best as possible in the face of the often amazingly destructive effector mechanisms of the immune system[5]. I do not think the lung is alone. Each organ is a complex combination of tissues, delicately balanced to perform a particular function: a function that can easily be compromised by the powerful effector mechanisms wielded by the immune system. Thus, tissues use all sorts of mechanisms to keep the cells and molecules of the immune system out until they need them and to control them when they arrive. If we accept that at least some immune responses can be initiated by tissue-derived signals that activate APCs, it is but a short step to suggest that there are also tissue-derived signals that educate those APCs in order to control the effector class of an immune response.

I can envisage two different ways in which a tissue can control the local effector class. First, it can directly educate its resident APCs such that those APCs, in turn, stimulate certain types of responses from T cells. There is direct evidence for such education in the gut (from analysis of APCs in Peyer's patches and mesenteric lymph nodes[6]), in the eye[7] (from analysis of the fluid of the anterior chamber and of the spleen APCs that this fluid drains to) and in the lung[5] (from analysis of lung macrophages). The tissue-specific education could alter the APCs either before activation (for example, Langerhans cells 'know' that they are in the skin before they become activated to migrate to a local node) or after. The second way a tissue could communicate class-specific instructions to the immune system would be to invite the immigration and residency of particular populations of 'innate' lymphocytes, such as the skin-resident γδ T cells (known as dendritic epidermal T cells) found in mouse and cattle skin[8], the γδ intraepithelial lymphocytes of the gut[9], the NK T cells found in human and mouse liver[10], the decidual NK cells found in human, mouse and equine placentas[11], and the B1 B cells found (in small numbers) in spleen and (in larger numbers) in the peritoneal cavity[10]. Many of these cells seem to be tuned to recognize stress-induced 'self' molecules rather than foreign pathogens. What is their function? Perhaps it is to help heal the tissue (such as with epidermal growth factor made by the dendritic epidermal T cell) or to ensure that a local immune response is shifted to the appropriate effector class to clear a pathogen without doing excess damage to the tissue itself.



Chris Sharp

Both injured or dying cells and pathogen-associated molecular patterns function as alarms that trigger the immune system into action. Collectively, they can be classified as damage-associated molecular patterns.

Complexity

How many different types of immune responses are there? We tend to read about T helper type 1 (T_H1), T_H2, T_H3 (though it is often called Tr1) and, more recently, T_H-17. But there are likely to be many more. If we describe a T_H1 response in terms of the signals and cytokines — such as interferon-γ (IFN-γ) and interleukin 12 (IL-12) — that produce activated CTLs, NK cells, mac-

rophages and complement-fixing antibodies (IgG2a in mouse); a T_H2 response (IL-4, IL-5) in terms of eosinophils and basophils plus IgG1 and IgE antibodies; and a T_H3 response (TGF-β, IL-10) as IgA, then what about the cells and cytokines that elicit IgG3? Or IgG2b or IgG2c? And how often do we even measure these responses?

Most labs (mine included) make the same appalling mistake. We tend to have a set of assays we routinely use and another set of assays that we do not use. So when we do something to an animal (for example, feed it an antigen), and notice that, by our assays, the immune response goes down (no more IFN-γ or T cell proliferation, or killing activity, or delayed-type hypersensitivity), we call it 'tolerance'. Then we transfer cells from that animal to another, find that the recipient also does not respond in our limited set of assays, and call it 'suppression' or 'regulation'. What we have not noticed is that both the original animal and the new recipient are responding ('behind our backs', as it were) in ways that we are not measuring (for example, making transforming growth factor (TGF-β) and IgA). This is not tolerance or suppression. It is simply a switch of effector class. People who work on oral vaccines (for example, the polio vaccine administered on a sugar cube) call this 'oral vaccination', whereas people working on autoimmunity call it 'oral tolerance'. Although the names are different, the response is the same: T helper cells making IL-4, IL-5, IL-10 and TGF-β, and B cells making IgA. In the case of polio, a virus that usually infects us orally, the response to oral polio is protective, so we call it vaccination. In the case of experimental autoimmune encephalomyelitis, the response to oral myelin basic protein switches the delayed-type hypersensitivity–T_H1 response to an IgA response. Because the IgA response does not cause destruction in the brain, and because the investigators do not measure it in other ways, they miss it and call this 'tolerance' or 'T regulation'. The latter name is actually appropriate. This *is* regulation. It is regulation of the effector class of the response. Unfortunately, when most immunologists say 'T regulatory' they mean 'T suppressor' and they think these cells suppress everything — though they have measured hardly anything except the characteristics of the T_H1 response.

If we are to understand the immune system, we need to acknowledge, even welcome, its complexity and measure the various ways that it manifests. We need to stop assuming that simply measuring IL-4 versus IFN-γ, or graft rejection versus non-rejection, or proliferation or IL-2 production, is a complete representation of immune effector classes.

Does it matter? A famous transplantation immunologist from Oxford once asked me if I would care whether a tolerance protocol managed to prevent the rejection of a kidney by inducing true deletional tolerance or by inducing regulatory T cells. The answer is yes, I do care. I care very much! We have no idea how stable 'regulation' is. Suppose that my kidney patient gets a bacterial or viral infection that cross-reacts with some of the kidney antigens? Do I know that the 'regulatory' cells maintaining the kidney won't now stop 'regulating'? Do I know their lifespan? Their span of activity? Their specificity? Do I know whether, in the process of 'regulating' a response to APCs presenting graft antigens, they will not also 'regulate' a response to an infection in the graft? Do we really want to transplant an organ that, by virtue of the mechanism that stops its rejection, is rendered immunologically unprotectable? I think it matters.

We need to think about all the aspects of an immune response, to measure as many of them as we can and to check those measurements in as many ways as possible. The technology to do this is arriving and we should not stick with the simplistic combinations of measures we have tended to use thus far.

Finally, the complexity does not stop with the cells of the immune system and the tissues they interact with. We are just beginning to scratch the surface of the communication between our commensals and us. We are an environment to an uncountable number of symbiotic, commensal and pathogenic organisms, each of which has had evolutionary time to learn how to use and misuse our immune system. As we expand our picture of the immune system from an army of lymphocytes patrolling the body for foreigners to an integrated group of communicating tissues, all working to maintain tissue integrity and health, we will necessarily need to include the signals from the non-self organisms that take advantage of that health or that help maintain it.

1. Janeway, C.A., Jr. *Immunol. Today* **13**, 11–16 (1992).
2. Matzinger, P. *Annu. Rev. Immunol.* **12**, 991–1045 (1994).
3. Matzinger, P. *Science* **296**, 301–305 (2002).
4. Seong, S. & Matzinger, P. *Nat. Rev. Immunol.* **4**, 469–478 (2004).
5. Takabayshi, K. *et al. Immunity* **24**, 475–487 (2006).
6. Johansson, C. & Kelsall, B.L. *Semin. Immunol.* **17**, 284–294 (2005).
7. Zamiri, P., Masli, S., Streilein, J.W. & Taylor, A.W. *Invest. Ophthalmol. Vis. Sci.* **47**, 3912–3918 (2006).
8. Havran, W.L., Jameson, J.M. & Witherden, D.A. *Am. J. Physiol. Gastrointest. Liver Physiol.* **289**, G627–G630 (2005).
9. Hayday, A., Theodoridis, E., Ramsburg, E. & Shires, J. *Nat. Immunol.* **2**, 997–1003 (2001).
10. Bendelac, A., Bonneville, M. & Kearney, J.F. *Nat. Rev. Immunol.* **1**, 177–186 (2001).
11. Moffett, A. & Loke, C. *Nat. Rev. Immunol.* **6**, 584–594 (2006).