

## ESSAY

# Tissue-based class control: the other side of tolerance

Polly Matzinger and Tirumalai Kamala

**Abstract** | In this Essay, we offer a new perspective on how immune responses are regulated. We do not cover how they are turned on and off, but focus instead on the second major aspect of an immune response: the control of effector class. Although it is generally thought that the class of an immune response is tailored to fit the invading pathogen, we suggest here that it is primarily tailored to fit the tissue in which the response occurs. To this end, we cover such topics as the nature of T helper ( $T_H$ ) cell subsets (current and yet to be discovered), the nature of privileged sites, the difference between oral tolerance and oral vaccination, why the route of immunization matters, whether the  $T_H1$ -type response is really the immune system's primary defense, and whether there might be a different role for some regulatory T cells.

When confronted with a potential threat, the immune system faces two decisions: first, whether to respond or not, and second, what kind of response to make. The first decision has fascinated immunologists for decades, sparking several theories and much experimental work, most of which rests on the assumption that the immune system responds to 'foreign' antigens, and that maintaining self tolerance is a matter of controlling autoreactive T and B cells. But dealing with autoreactive lymphocytes is only half of the problem. Even in the absence of any autoreactivity, the wrong immune effector class can completely destroy a tissue<sup>1–5</sup>.

The control of effector class, however, has had little theoretical input. Students generally learn that the immune system matches the effector class to the pathogen that it is fighting (for example, making IgE against worms, and cytotoxic T lymphocytes (CTLs) against viruses and intracellular bacteria). But it is not easy to see how the immune system could discriminate between worms, viruses or intracellular versus extracellular bacteria, as T cell receptors bind peptide–MHC complexes; B cell receptors bind small epitopes on proteins, carbohydrates and lipids that are present in most living

organisms; and the 'innate' receptors, such as the Toll-like receptors (TLRs) and NOD-like receptors (NLRs), are so promiscuous that they don't distinguish between ligands from different phyla, or between pathogen-derived and self-derived alarm signals<sup>6–9</sup>. Although current data suggest that TLR5 and NOD2 (nucleotide-binding oligomerization domain protein 2) may be fairly specific, it would be difficult to use even these receptors to make decisions about effector class. NOD2 does not distinguish between intracellular and extracellular bacteria, as it binds muramyl dipeptide, a component of almost all bacterial cell walls<sup>10</sup>. Similarly, TLR5 binds the flagella of both intracellular pathogens (such as *Listeria monocytogenes*) and extracellular pathogens (such as *Escherichia coli* and *Pseudomonas aeruginosa*)<sup>11</sup>, whereas it does not bind the flagella of several other important pathogens (including *Helicobacter pylori*, *Staphylococcus aureus* and *Campylobacter jejuni*)<sup>11</sup>.

So, what controls the effector class of an immune response? The idea that it might be the tissues, rather than the immune system, has grown slowly over the 13 years that we have been studying immunity from the perspective of the danger model<sup>12,13</sup>. Initially,

the model did not offer any clues as to how one effector class might be chosen over another, as it was designed to cover only the immune system's first decision (whether to respond or not). It proposed that perturbed tissues initiate immune responses by sending alarm signals that activate local antigen-presenting cells (APCs), whereas healthy tissues display their own antigens or allow 'resting' APCs to display those antigens to induce peripheral tolerance. In effect, this model suggested that turning immune responses on or off was the prerogative of the tissues. It takes only a small step to suggest that tissues may also control the effector class, such that the class of an immune response is tailored to the tissue in which it occurs, rather than to the invading pathogen. The basics of this idea were outlined in two earlier articles<sup>12,14</sup>. In this Essay, we describe the idea more fully, suggesting mechanisms by which tissues could carry out this function, describing some well-known immunological phenomena in light of this view, and pointing out the possibility that a complete definition of the immune system should perhaps include every tissue in the body.

## Class and T helper cell subsets

Let us start by defining what we mean by immune effector class. Although the term "class" has historically been used to define different antibody isotypes (such as IgM and IgG), we prefer a definition that also includes the participating cells. Thus, each effector class combines a particular set of helper cells and the antibodies and effector cells that they promote. Currently, three main subclasses are generally accepted.  $T_H1$ -type responses consist of T cells that produce interleukin-2 (IL-2), interferon- $\gamma$  (IFN $\gamma$ ) and tumour necrosis factor (TNF), as well as B cells that make complement-fixing IgG antibodies, CTLs, activated natural killer (NK) cells and macrophages that produce free oxygen radicals.  $T_H2$ -type responses consist of T cells that produce IL-4, IL-5, IL-13 and IL-10, B cells making IgE and IgG1, macrophages that express arginase, and the influx of eosinophils.  $T_H17$ -type responses consist of T cells that produce IL-17 and the influx of neutrophils.

However, there have long been clues that this is an oversimplification. Human T cells, for example, often show non-classical cytokine expression patterns, and it took a long time to persuade researchers working with human cells to adopt the  $T_H1$ - and  $T_H2$ -type classifications. In mice, Kelso<sup>15</sup> found that micromanipulated single T cells do not stably make  $T_H1$ - or  $T_H2$ -type cytokine ‘cassettes’. She suggested that the  $T_H1$ - and  $T_H2$ -type patterns are only the extremes of a multidimensional continuum; that individual T cells normally make only a small number of stochastically produced cytokines; and that populations of T cells can diversify to produce any number of different cytokine combinations. More recently, O’Shea and Paul<sup>16</sup> proposed a somewhat similar scenario, but these views are far from universal. For example, Pulendran’s and Oppenheim’s groups found that dendritic cells (DCs) stimulated by *Porphyromonas gingivalis*-derived lipopolysaccharide (LPS)<sup>17</sup> or by eosinophil-derived neurotoxin<sup>18</sup> induce  $T_H$  cells that secrete IL-5 but not IL-4 (the signature  $T_H2$ -type cytokine). In addition, Prussin and colleagues<sup>19</sup> found two different subsets of  $T_H$  cells in atopic patients, some of which produce IL-5 but not IL-4. Nevertheless, rather than postulate the existence of a  $T_H$  cell that did not fit into the standard categories, all of these authors called these IL-5-producing cells “ $T_H2$  cells”.

We believe it is time to follow Kelso’s lead and stop forcing various kinds of immune responses into a few common categories. Although the  $T_H1/T_H2$  paradigm has been useful in establishing the concept that different sorts of  $T_H$  cells promote different classes of

response, it has limited our ability to recognize the potentially enormous diversity of immune responses. If we were to stop consigning  $T_H$  cells to a small group of numbered subsets, but instead name them by what they produce (as is done for  $T_H17$  cells) or by the responses they promote (as is done for follicular helper T cells), we would uncover the possibility that there are a large number of differentiation paths that  $T_H$  cells can take. We would suggest that each particular effector cell (such as each type of B cell, CTL, NK cell, macrophage, eosinophil, neutrophil and basophil) is controlled by a particular set of secreted and membrane-bound signals (from  $T_H$  cells and from other sources) and can be combined with any other effector cell to make a wide variety of carefully tailored immune responses.

Given the existence of such a variety of effectors, and the  $T_H$  cells that facilitate them, what determines the ultimate effector combination that arises in any particular immune response? We were drawn to the possibility that this is the responsibility of the tissues by two old immunological phenomena: immune-privileged sites and oral tolerance.

#### Immune-privileged sites

Immune-privileged sites are organs in which allogeneic transplants are not rejected. Neonatal hearts, for example, are rejected when transplanted under the skin or kidney capsules of adult recipients<sup>20</sup>, but survive indefinitely if placed into the anterior chamber of the eye<sup>21</sup>, the brain<sup>21</sup>, the testes<sup>22</sup> or the hamster cheek pouch<sup>23</sup>. These observations are generally interpreted as evidence that ‘privileged’ sites exclude<sup>24</sup>, disable<sup>25</sup> or suppress<sup>26</sup> immune cells.

There is, however, a world of difference between data and interpretations. The interpretation that immunity cannot occur in privileged sites makes little evolutionary sense. These tissues are wet, warm and full of nutrients. Without protection by the immune system, wouldn’t they promptly be exploited by pathogens? Luckily, other interpretations exist. Streilein’s work on the eye reveals a much more interesting picture.

The eye is a complex tissue, containing delicate specialized cells that cannot survive a full-blown  $T_H1$ -type or delayed-type hypersensitivity (DTH) response. It protects itself by a process Streilein called “anterior chamber-associated immune deviation”<sup>27</sup>, in which cells lining the anterior chamber secrete cytokines — transforming growth factor- $\beta$  (TGF $\beta$ ), vasoactive intestinal peptide (VIP) and  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ MSH) — that suppress  $T_H1$ -type and DTH responses and increase the activity of regulatory T ( $T_{Reg}$ ) cells<sup>28</sup> (TABLE 1).

Although this appears to be immune suppression, a closer look suggests something different. TGF $\beta$ , VIP and  $\alpha$ MSH all promote IgA production<sup>29–32</sup>, but IgA cannot reject a transplant. So if we measure ocular immunity only by transplant rejection, and ignore the perfectly functional IgA response, we call it “tolerance”, “deviation”, “suppression” or “regulation”. But this is none of those things. It is simply a class of response that protects the eye without destroying it.

The cells of the eye also express FAS ligand (also known as CD95L), which can trigger T cells through surface FAS (also known as CD95) to die by apoptosis<sup>25</sup>. Although initially interpreted as evidence that lymphocytes entering the eye are eliminated, newer evidence that  $T_H1$  cells express more FAS than  $T_H2$  cells<sup>33</sup> suggests that the eye ‘chooses’ what kinds of  $T_H$  cells it allows or excludes. We predict that the other privileged sites also exert similar control over the local class of immune response.

#### Oral tolerance and oral vaccination

Oral tolerance has been extensively studied in experimental autoimmune encephalomyelitis. This is an experimental system in which an animal that is immunized with a strong adjuvant plus a brain-derived protein, such as myelin basic protein (MBP), acquires an autoimmune disease mediated by  $T_H1$ -type,  $T_H17$ -type and DTH responses<sup>34</sup> that somewhat resembles multiple sclerosis in humans. Feeding the animal MBP prior to MBP immunization prevents disease and reduces T cell responses<sup>35,36</sup>. The T cells no longer proliferate in response to MBP or make IFN $\gamma$

Table 1 | Cytokines that tailor immune effector class in the eye and gut

Cytokine	DTH or $T_H1$ -type response	$T_{Reg}$ cell induction	IgA production
<i>In the eye</i>			
TGF $\beta$	↓ <sup>140</sup>	↑ <sup>141</sup>	↑ <sup>29,30</sup>
VIP	↓ <sup>142</sup>	↑ <sup>143</sup>	↑ <sup>31,32</sup>
$\alpha$ MSH	↓ <sup>144</sup>	↑ <sup>145</sup>	?
<i>In the gut*</i>			
TGF $\beta$	↓ <sup>140</sup>	↑ <sup>141</sup>	↑ <sup>29,30</sup>
VIP	↓ <sup>142</sup>	↑ <sup>143</sup>	↑ <sup>31,32</sup>
TSLP	↓	↑ <sup>146</sup>	↑ <sup>147†</sup>
Vitamin A (retinoic acid)	↓ <sup>148,149</sup>	↑ <sup>119,150,151</sup>	↑ <sup>57</sup>

DTH, delayed-type hypersensitivity;  $\alpha$ MSH,  $\alpha$ -melanocyte stimulating hormone; TGF $\beta$ , transforming growth factor- $\beta$ ;  $T_{Reg}$ , regulatory T; TSLP, thymic stromal lymphopoietin; VIP, vasoactive intestinal peptide.

\*There are other molecules secreted by gut epithelial cells (such as APRIL (a proliferation-inducing ligand; also known as TNFSF13) and BAFF (B cell-activating factor; also known as TNFSF13B) that are not as well studied but which are likely also to influence the class of the immune response. †TSLP promotes IgA indirectly by promoting APRIL and interleukin-10 production.

or TNF and, when transferred into another mouse, these T cells suppress the recipient's autoimmune response. The interpretation of these results, that orally administered antigen generates tolerance and  $T_{\text{H}}^{\text{Reg}}$  cells, has spurred a novel treatment for allergy known as sublingual immunotherapy<sup>37</sup>, in which patients apply small amounts of allergen sublingually each day.

But what about oral vaccination, which can elicit protective immunity against poliovirus in humans<sup>38</sup> and against rabies virus in raccoons and coyotes<sup>39</sup>? What is the difference between oral vaccination and oral tolerance? In many cases, very little. Oral administration of antigen in mice elicits at least three different kinds of response. When given in large doses, it can induce systemic deletional tolerance<sup>40,41</sup> (presumably because enough antigen leaks into the circulation to be presented by resting DCs), as well as a local IgA response<sup>42</sup>. The addition of various adjuvants converts this into a systemic response that includes CTLs and IgG<sup>43</sup>. Lower doses of antigen can lead to the activation of  $T_{\text{H}}3$  cells that produce IL-4, IL-5, IL-10 and TGF $\beta$  and promote the production of IgA<sup>42,44</sup>. Because the  $T_{\text{H}}3$  cells can suppress inflammatory mechanisms through their secretion of IL-10 and TGF $\beta$ , and because the presence of IgA is rarely assessed, this type of response is also often labelled as tolerance. But this is not tolerance. It is simply a switch to an immune response that is appropriate for the intestinal environment.

How does the gut promote the IgA response? Intestinal epithelial cells express TLRs<sup>45</sup> and secrete cytokines<sup>46</sup> (TABLE 1). Some of these cytokines (including TGF $\beta$  and VIP) are similar to those produced in the eye, others (such as thymic stromal lymphopoietin (TSLP)) are unique to the gut, and some factors (such as vitamins A and D) are acquired in the diet and modified for use. All of these factors shape the immune response (TABLE 1). For example, TGF $\beta$ , TSLP, vitamin A and vitamin D have been shown to suppress  $T_{\text{H}}1$ -type and DTH responses and promote the production of IgA. Thus, the gut seems to promote the antibody subclass (IgA) that is locally most useful, while simultaneously preventing destructive  $T_{\text{H}}1$ -type and DTH responses.

### Tissue-appropriate immunity

Why would a tissue suppress DTH and  $T_{\text{H}}1$ -type responses? Why not make many different effector classes to ensure pathogen clearance? The reason is that these mechanisms are terribly destructive. TNF and IFN $\gamma$  induce cell death<sup>47,48</sup>; IL-17 recruits

### Box 1 | Class control by organs, tissues or regions?

Is it organs or tissues that control the immune response? On the one hand, one could argue that the function of each organ dictates that it should encourage particular types of response and discourage others. But are organs homogeneous in their needs? For example, the skin has a barrier function that may require certain types of immune functions, but the dermis and epidermis are not the same. They have different populations of antigen-presenting cells (APCs) and might promote different immune response classes. The jejunum and ileum, although comprising parts of the small intestine, have their own subsets of microflora and may need somewhat different types of immune protection. Even within these intestinal regions, the epithelial microenvironments are different. In the villus crypts, where most epithelial cell division occurs and where bacterial infection would be particularly hazardous, Paneth cells produce large amounts of antimicrobial peptides. Further up the villus, the epithelial cells take on more of their own protection, expressing Toll-like receptors and producing different types of bactericidal molecules.

One could argue, then, that each cell type has the ability to produce immune protective and immune-modulating signals, and that this implies that control of the immune response lies at the level of the cell. However, the same cell type might behave differently in different organs. For example, the vascular endothelium is unlikely to be the same in the lungs, liver, heart, skin and kidneys. Will it communicate differently in those different sites with the cells of the immune system?

At the moment we don't have answers to these questions. We don't know what comprises a minimum tissue 'unit' that communicates with the immune system. For that reason, we use the word "tissue" to define a local mixture of tissue cells that communicate with each other and with the bone marrow-derived cells that constitute the rest of the immune system. In some cases, a local tissue might also communicate systemically with other tissues to help define the initiation, longevity and effector class of an immune response.

neutrophils; CTLs and NK cells kill target cells directly or through antibody-dependent cell-mediated cytotoxicity<sup>49,50</sup>; macrophages release oxygen radicals<sup>51</sup>; and complement drills holes in cell membranes<sup>52</sup>. These are devastating weapons!

The eye and gut are not alone in being susceptible to damage by these powerful responses. Each organ is made of an intricate combination of tissues (BOX 1), precisely tuned to perform particular functions that can easily be compromised by destructive effector mechanisms. For example, strong  $T_{\text{H}}1$ -type responses can destroy the placenta<sup>1,53</sup>, pancreatic islets<sup>3</sup>, skin<sup>1</sup>, eye<sup>4</sup>, brain<sup>54</sup> and small intestine<sup>2</sup>. So most tissues are likely to have mechanisms to avoid such destruction while promoting appropriate local immunity.

How might tissues communicate their preferences to local and circulating cells of the immune system? First, the local tissue can produce (or modify) cytokines, chemokines and other communicating molecules (for example, antimicrobial peptides such as LL37 (REF. 55); neuroactive molecules such as VIP and noradrenaline<sup>56</sup>; or vitamins such as vitamins A<sup>57</sup> and D<sup>58</sup>). These factors can influence tissue-resident APCs to promote a certain effector class (or classes) while discouraging others. They can also affect the entry and exit of innate and adaptive immune cells, and govern what these cells do in the local environment. For example, fluid from the eye's anterior chamber can induce peritoneal macrophages to suppress

DTH responses<sup>59</sup> and enhance  $T_{\text{H}}2$ -type responses<sup>60</sup>. In the gut, TGF $\beta$  acts as a switch factor that induces B cells to produce IgA<sup>61</sup>, and vitamin D helps to recruit  $T_{\text{H}}2$  cells rather than  $T_{\text{H}}1$  cells by promoting the production of CC-chemokine ligand 22 (CCL22)<sup>62</sup>, which recruits CC-chemokine receptor 4 (CCR4)<sup>+</sup>  $T_{\text{H}}2$  cells<sup>63</sup>. In the skin, locally produced vitamin D can suppress local DTH responses<sup>64</sup>.

Second, many tissues have resident T cells that respond to stress-induced self molecules rather than foreign antigens. The gut has intraepithelial  $\alpha\beta$  T cells<sup>65</sup> and  $\gamma\delta$  T cells<sup>66</sup>, as well as mucosa-associated invariant T (MAIT) cells<sup>67</sup>. These cells respond to the stress-induced self molecules RAE1 (retinoic acid early-inducible protein 1) and MR1 (MHC class I related) in mice, and to MICA (MHC class I polypeptide-related sequence A), MICB and MR1 in humans<sup>68</sup>. Similarly, up to 40% of liver-resident T cells are NKT cells that recognize several lipid molecules presented by the stress-induced antigen-presenting glycoprotein CD1d<sup>69</sup>. Mouse and cattle skin contains dendritic epidermal  $\gamma\delta$  T cells (DETCs), which respond to RAE1 (REF. 70) and help to heal the skin by making keratinocyte growth factor<sup>71</sup>, IL-2 and IFN $\gamma$ <sup>72</sup>. The purpose of these tissue-resident cells seems to be to survey the tissues they reside in for signs of stress and to maintain the health of these tissues. The cells may achieve this by making molecules that are important for healing and/or by producing cytokines (and probably

chemokines, endogenous danger signals and neuropeptides) that promote the class of immune response appropriate for that tissue at that time.

Third, neuronal signals may contribute to tissue-specific class control. Neuropeptides can influence effector class<sup>73</sup>, and many leukocytes express neuropeptide receptors that were previously thought to be restricted to the nervous system<sup>74–78</sup>. Overall, tissues seem to have multiple ways of communicating with the immune system and promoting appropriate local immune functions. We assume that each tissue has a particular set of effectors that it prefers. IgA, for example, is appropriate for the eye<sup>79</sup>, the gut and other mucosal surfaces, but may not be the right effector for the brain, which would have its own preferred response class.

### The three phases of an immune response

If T<sub>H</sub>1-type, T<sub>H</sub>17-type and DTH responses are so harmful, why have they evolved? One possibility is that tissues that regenerate easily (such as the skin and liver) can tolerate the damage, whereas others (including the eye, brain and pancreatic islets) might not. Thus, a DTH response in a non-regenerative tissue might be a case of the right action in the wrong place.

Another possibility is that DTH is the immunological counterpart of frostbite. On exposure to cold, capillaries dilate to keep extremities warm, but with intense cold or long exposure the capillaries constrict, causing loss of extremities while preserving core temperature. Perhaps the immune system's response is similarly biphasic. It first uses less destructive immune mechanisms to deal with adversity in a way that maintains the health of all tissues. But if that doesn't work, it switches to a second phase of destructive responses, sacrificing some tissues to preserve life.

In fact, there could be three phases. In phase one, the tissue summons innate cells for clean up and repair. If this isn't sufficient, it recruits the adaptive immune system, which attempts to clear the problem with a locally tailored effector class. And if that doesn't work, the tissue brings in the highly destructive T<sub>H</sub>1-type, T<sub>H</sub>17-type and DTH responses. DTH responses might destroy the eye while clearing an ocular infection<sup>80</sup>, but they ensure that the individual survives. In the gut, DTH responses may cause temporary flattening of gut villi and result in diarrhoea, but villi regenerate quickly. And, although a T<sub>H</sub>1-type response may cause a fetus to abort while clearing an infection, it saves the mother's life.

Nevertheless, such destruction is a tremendous price to pay for good health and should only be summoned when necessary. How does a tissue determine when to switch from one class of immune response to another?

### Controlling the switch

When considering what might control the switch between the phases of an immune response, we begin with three assumptions. The first is that tissues 'educate' resident APCs (and incoming T and B cells) to promote certain types of immune response and suppress others<sup>81–86</sup>. Although it has been suggested that there are many subsets of DCs because of the need to distinguish many different pathogens<sup>16</sup>, these subsets might also correlate with the different kinds of tissues that need protection (for example, Langerhans cells can be distinguished from dermal DCs in unperturbed skin). Furthermore, DCs remember their origins. DCs that migrate from the gut to mesenteric lymph nodes induce T cells to express gut-homing receptors (such as CCR9)<sup>87</sup> and produce cytokines (including IL-4, IL-10 and TGFβ) that suppress T<sub>H</sub>1-type responses and promote IgA production<sup>41,61,81,88</sup>, whereas DCs that leave damaged skin induce T cells to express skin-homing receptors (namely α4β1 integrin, CCR4 and CCR10)<sup>89,90</sup>.

We predict that a thorough analysis will show that each tissue imparts specific instructions that result in different populations of T<sub>H</sub> cells promoting different combinations of effectors, each appropriate for that particular injured or infected tissue. For example, three different T<sub>H</sub> cell subsets protect three different organs: CD62L<sup>+</sup> T cells protect islets from diabetes, CD25<sup>+</sup> T cells protect the stomach from gastritis, and CD45RB<sup>low</sup> T cells protect the gut from colitis<sup>91</sup>.

The instructions that tissues pass to T cells might be communicated through tissue-derived molecules, which could influence local or newly entering T cells directly or which could be transported to the T cells by APCs. Alternatively, the signals might be produced by APCs as a consequence of the education that they received from their local tissue. If antigen recognition delivers 'signal one' and co-stimulation delivers 'signal two', then tissue-influenced signals governing the ensuing effector class could be called 'signal three'.

The second assumption we make is that when signal three is absent, the default action is a T<sub>H</sub>1-type or DTH response. If a tissue becomes so damaged that it cannot deliver APC-educating signals, the backup response

is probably necessary. This fits with the common findings that T cells stimulated by DCs generated without tissue-derived signals (such as DCs generated in plastic dishes) or by CD3-specific antibody tend to produce T<sub>H</sub>0- or T<sub>H</sub>1-type responses, whereas T cells stimulated by DCs from mesenteric lymph nodes or Peyer's patches produce IL-4, IL-10 and TGFβ<sup>88,92</sup>. Thus, tissue-derived signals serve as checkpoints to prevent the destructive default backup response.

Third, we assume that APCs carrying tissue instructions to draining lymph nodes survive for a while and are then replaced by new APCs. Their experimentally deduced functional lifespans range from 3 days<sup>93</sup> to 3 weeks<sup>94</sup>.

Given these assumptions, there are at least two non-exclusive possibilities for how tissues might manage the switch from locally tailored immune responses to destructive backup responses: one based on time, the other on signal strength.

**Time-dependent class switch.** When activated by exogenous or endogenous<sup>6,95,96</sup> alarm signals, tissue-resident APCs migrate to the draining lymph nodes and are replaced by new APC precursors. If the immune

**Figure 1 | A model for tissue-based class control of immune responses.** **a** | Resting tissues educate local antigen-presenting cells (APCs). **b** | Following an insult (such as an injury or infection), the APCs leave the tissue to stimulate naive T cells to make tissue-educated responses. **c** | If the innate immune response clears the infection (or injured tissue), the tissue heals and educates newly arriving APCs. An adaptive immune response is not needed and ceases. **d** | If the innate immune response does not stop the infection, then tissue-educated adaptive immune responses are initiated. If these clear the pathogen, then the tissue heals. **e** | If the tissue-educated adaptive immune response cannot resolve the infection, then a second wave of newly entering APCs will be activated in a local tissue environment that now contains more extensive damage. The new APCs may be properly educated or they may not be (because the high level of damage would result in fewer signals from the tissue). If not, they will leave the tissue and stimulate the emergency backup response. **f** | If the backup response clears the pathogen, then the tissue heals, but with some scarring or fibrosis occurring. **g** | If the initial insult is severe, the local APCs leave the tissue without receiving a complete education. This could be because the severely damaged tissues cannot provide the right signals or because the tissue provides signals that override the original education. These APCs launch the immediate backup response. DTH, delayed-type hypersensitivity; TCR, T cell receptor; T<sub>H</sub>1, T helper 1.





response elicited by the original wave of tissue-resident APCs clears the infection, the new APC precursors that migrate to the healing or healed tissue will become the new resident tissue-educated APCs. If, however, the infection persists, this second wave of APCs may be activated before they are educated. When these uneducated APCs migrate to the draining lymphoid tissue, they will elicit the default backup  $T_H1$ -type or DTH response. In this scenario, the backup mechanism occurs only because the initial tissue-oriented response has failed (FIG. 1).

**Damage- or stimulus-dependent class switch.** Under conditions of severe infection<sup>97</sup>, cell stress or destruction<sup>8</sup>, or in the presence of noxious adjuvants<sup>28</sup>, the local tissue may need to promote a strong backup response right from the start or may simply have difficulty sending educating signals. APCs migrating from the tissue without having had the proper final set of instructions will, therefore, induce the backup response. Later, when most of the infection has been cleared and the healing tissue is once again able to send educating signals, the response will revert to the tissue-oriented response<sup>98</sup>. This might account for the occasional late appearance of less noxious responses<sup>99</sup> (FIG. 1).

These are not mutually exclusive scenarios. There might be situations in which memory T cells influence APCs after they arrive at the draining lymph node and counteract the tissue-derived instructions that the APCs arrived with. For example, T cells induced by oral immunization can enter popliteal lymph nodes and re-educate DCs to promote  $T_H2$ - or  $T_H3$ -type responses rather than  $T_H1$ -type responses<sup>83,92</sup>. There might be times when the signals that APCs receive through their innate receptors are so strong that they overcome the tissue-educating signals. There might also be positive or negative feedback loops. For example, DCs that promote  $T_H1$ -type or DTH responses make IL-12, which stimulates NK cells. In turn, activated NK cells can rapidly kill activated DCs<sup>100</sup>, ensuring that a  $T_H1$ -type or DTH response doesn't last long. And when the damage gets too great, the resulting hypoxia can downregulate the destructive immune response and switch it to another class<sup>101</sup>.

### What about the pathogens?

One could argue that the idea that tissues promote their own particular types of response, irrespective of the infecting agent, goes against a wealth of data supporting

the textbook consensus that the immune system tailors its responses to the pathogen (by initiating CTL responses to viruses and intracellular bacteria, and  $T_H2$ -type responses to extracellular bacteria and worms)<sup>16,102</sup>. However, the evidence is not as clear as the textbooks would like to us to believe.

First, the view that the immune system mobilizes CTLs to clear viruses and IgE to eliminate worms is rather oversimplified. Most viruses elicit antibody responses, and the isotypes vary with the infection site. Measles and rubella viruses, for example, inhabit the skin<sup>103</sup> and elicit mostly IgG1, IgG3 and IgG4 (REF. 104), but rotavirus and influenza virus, which inhabit the gut and the lungs, respectively, induce strong IgA responses<sup>105</sup> (which can be more important than CTLs for protection from reinfection<sup>106</sup>). Unfortunately these local tissue responses are often missed, either because they simply aren't measured (for example, TGF $\beta$  and secretory IgA are often left out of standard tests because they are difficult to measure) or because the relevant B and T cells home to distinctly local sites using specific chemokine receptors and adhesion molecules<sup>107,108</sup>, and discharge their antibodies and cytokines into local secretions rather than serum<sup>109</sup>.

“ Tissues are not simply passive recipients of immune protection ”

What about the idea that  $T_H2$ -type responses are the best mechanism to clear helminths? In mice infected with schistosomes, treatment with antibody specific for IgE actually causes a reduction in worm burden<sup>110</sup>, and worm numbers do not differ between IL-4-deficient and wild-type mice<sup>111</sup>. Furthermore, IL-4-deficient mice have lower burdens of *Onchocerca* microfilariae and greater resistance to reinfection than wild-type mice<sup>112</sup>. In human studies, people living in *Schistosoma mansoni* endemic areas who remain clinically uninfected make strong IFN $\gamma$  responses to worm antigens, whereas those who maintain chronic low worm burdens make IL-4 (REFS 113, 114). This suggests that an IFN $\gamma$ -associated response clears the worms and prevents reinfection, whereas  $T_H2$  cell functions instead allow the worms to establish low-level colonization and produce more worms without doing serious damage. One could ask,

therefore, if the IgE responses reported against worms might be due to instructions from the worm, rather than decisions by the immune system.

When studying immunity to a pathogen, it is not always clear whose agenda we are studying. Viruses are known to have evolved all sorts of mechanisms to subvert or modulate immune responses<sup>115</sup>, and we should not expect less from pathogens with larger and more complex genomes<sup>116</sup>. Their survival depends on it and they have had evolutionary time to devise the mechanisms. BOX 2 gives a few examples of the strategies used by parasites and worms to modulate immune responses in their favour.

### The influence of history

If tissues and their resident immune cells tend to promote immune response classes other than the  $T_H1$ -type or DTH response, why have immunologists thought of the  $T_H1$ -type response as the 'normal' response for so long? This may be a historical accident. Before we had *in vitro* culture systems and other laboratory tools, there were two main ways of measuring cellular (as opposed to humoral) immunity: the tuberculosis skin DTH test and graft rejection. Later, as we developed *in vitro* correlates of such cellular immunity, we looked for components that were part of these responses. For example, we generated assays for CTLs and NK cells, for macrophages that produce oxygen radicals, for T cells that produce TNF and IFN $\gamma$ , and for antibodies that fix complement. In other words, we measured things that kill! If anyone wondered why immune responses should be so destructive, they probably assumed that this is the only way to fight pathogens and that the collateral damage is simply the price we pay.

Because what we think influences what we do, we also geared our model systems to generate these responses. We found culture conditions to promote them *in vitro* and adjuvants that elicit them *in vivo* (is it any surprise that our 'best' adjuvant for cellular immunity, complete Freund's adjuvant, contains mycobacteria?). We named the CD4<sup>+</sup> T cells that enhance them  $T_H1$  cells, and when we discovered that IgE production and allergy seem to be driven by a different kind of helper cell, we named them  $T_H2$  cells and called their suppressive effect on  $T_H1$  cell responses 'immune deviation'. When we discovered that orally administered antigen elicits  $T_H3$  cell responses, which involve  $T_H$  cells making TGF $\beta$  and IL-10 and promoting IgA production, we

## Box 2 | Class control by pathogens

Viruses are known to have a plethora of mechanisms to influence and/or avoid the immune system<sup>115</sup>. Other organisms have not been as extensively studied, but some data are beginning to emerge. Some pathogens might choose to reside in tissues that not only offer good shelter and nutrition, but that also promote an effector class that is unable to effectively clear that parasite. Pathogens can also generate their own immune-influencing signals to exploit host defense strategies to their advantage. *Leishmania* parasites are an example. Sandflies taking a blood meal on mammalian skin induce a typical wound healing response that summons 'alternatively activated' macrophages that express high levels of arginase<sup>130</sup>. This is precisely what *Leishmania* parasites need: readily available macrophages that they can infect to start the next stage of their life cycle. How does the macrophage-associated arginase help in this process? Catabolism of arginine involves two enzymes that compete with each other. Inducible nitric oxide synthase (iNOS) generates nitric oxide, which effectively kills intracellular *Leishmania*, whereas arginase catabolizes arginine to ornithine, a precursor of essential nutrients for *Leishmania*<sup>131</sup>. *Leishmania* parasites influence events even more in their favour by inducing the sandfly to secrete promastigote secretory gel (PSG), a potent inducer of arginase-expressing macrophages<sup>132</sup>. The more parasites harboured by the sandfly, the more PSG in its midgut and the more it deposits in the skin when it bites. Thus, the process by which *Leishmania* are deposited into host skin during a natural infection induces precisely the responses that are most likely to maximize *Leishmania* survival and propagation, namely the migration to the site of infection of macrophages that synthesize the nutritional compounds that *Leishmania* require.

Mycobacteria also have immune-subverting effects. Recent studies<sup>133</sup> on early events in the establishment of mycobacterial infections have reversed long-standing assumptions about the purpose of the granuloma, one of the most ancient host defense strategies by which multicellular organisms wall off infectious agents and prevent their spread through the body<sup>134</sup>. Surprisingly, granulomas form rapidly during infection with virulent mycobacteria and have greater levels of macrophage recruitment, motility and apoptosis than those that form during infection with non-virulent mycobacteria (which are poorly formed and result in attenuated infections). The result of the accelerated granuloma formation with virulent mycobacteria is early dissemination of the infection, through the release of infected macrophages from the primary granulomas and production of secondary granulomas at distal sites<sup>133</sup>. Thus, the virulent mycobacterium converts an evolutionarily ancient form of host defense into a convenient and pliant tool that enables its survival and more efficient propagation.

*Bordetella bronchiseptica*<sup>135</sup>, *Schistosoma mansoni*<sup>136–138</sup> and *Fasciola hepatica*<sup>9</sup> express molecules that promote T helper 2 cell responses, whereas Lewis antigen-expressing *Helicobacter pylori*<sup>139</sup> specifically block T helper 1 cell differentiation, presumably because such strategies work to their advantage. These strategies are likely to be only the tip of the iceberg, and we will find many more as we study the relationships between pathogens, commensals, symbionts and their hosts.

mostly ignored them, or focused instead on their ability to suppress the  $T_H1$ -type inflammatory response and called them  $T_{Reg}1$  cells or  $T_{Reg}$  cells<sup>117–120</sup>.

Although  $T_{Reg}$  cells were originally thought to be autoreactive T cells that are educated in the thymus<sup>121</sup> to become suppressors<sup>122,123</sup>, there are now known to be several subsets. There are natural and induced  $T_{Reg}$  cells, thymic and peripheral  $T_{Reg}$  cells and self-reactive and non-self-reactive  $T_{Reg}$  cells. There are  $T_{Reg}$  cells induced by culture in the absence of co-stimulatory molecules and those induced in the presence of co-inhibitory molecules or other molecules such as TGF $\beta$ , vitamin D, retinoic acid or antibodies specific for CD40 ligand. There are  $T_{Reg}$  cells that make TGF $\beta$  or IL-10 or neither. And there have been endless discussions about which ones are the 'real'  $T_{Reg}$  cells<sup>124</sup>. We would suggest that many of these  $T_{Reg}$  cells are actually misunderstood memory helper T cells. In an unimmunized animal, they

will have been stimulated mostly by antigens entering through the nose, mouth and intestine. To help to promote appropriate responses for these mucosal tissues they may also need to suppress other classes of response. It would not be surprising, therefore, to discover that many CD25<sup>+</sup> memory  $T_H$  cells from a normal mouse can suppress IFN $\gamma$  or TNF production, graft rejection or CTL activity.

One might ask why our postulated helper activity of tissue-specific  $T_{Reg}$  cells has not been observed. The main reason is that  $T_{Reg}$  cells are not often tested for helper functions. More than 99% of the work on  $T_{Reg}$  cells uses assays that measure only their ability to suppress  $T_H1$ -type functions<sup>125</sup>. If we don't measure the mucosal memory response, we will mistakenly call this tolerance, suppression or regulation. In fact, in two studies in which intestinal antibody production was measured, forkhead box P3 (FOXP3)<sup>+</sup> cells were shown to become helper cells for the production of IgA<sup>126,127</sup>.

We are all guilty of this. Out of all of the ways to evaluate immune responses, each laboratory tends to use only a small subset. So when we do something (such as feed antigen to animals, or add cytokines or antibodies to *in vitro* cultures) and notice that the immune response we are measuring decreases, we call it tolerance. Then when we transfer cells from that animal to another, or add them to other cells *in vitro*, and find that the recipient also fails to respond in our limited set of assays, we call it suppression. But we don't notice that, unobserved, another type of response is increasing. If, for example, we measured  $T_H2$  cells only by their ability to suppress  $T_H1$ -type responses, we would miss their capacity to promote IgE and IgG1 production and their role in allergy and asthma. This is not really suppression, but a switch of effector class. Perhaps, if we were to begin measuring the helper functions of  $T_{Reg}$  cells, we might find a new range of tools to help us modulate immunity.

We suggest that it is time to discard the idea that the destructive  $T_H1$ -type or DTH response is the immune system's core mechanism. When someone tells us that the  $T_H1$ -type response is the "natural" response in their experimental system, we need to ask, "what adjuvant are you using, what dose of antigen are you using, where are you injecting it and what other responses are you missing?"

## A few final thoughts

Although the body must clear some pathogens, or at least keep them in their place, our commensal microflora must also be maintained and our tissues kept healthy in the process. To accomplish this, the immune system does not use a set of rigidly defined  $T_H1$ ,  $T_H2$  or  $T_H17$  cells, but a wide variety of  $T_H$  cells that respond to signals from their environment to mount a carefully balanced response to adversity. Some of these cells are tissue resident and some circulate. Some have genetically defined invariant T cell receptors, whereas others are somatically generated in each individual. Each of these  $T_H$  cells associates with various groups of B cells, macrophages, cytotoxic cells, neutrophils, eosinophils, basophils and tissue cells to tailor the response to the local milieu and the pathogen.

In fact, we should perhaps redefine the immune system to include every tissue in the body. Tissues are not simply passive recipients of immune protection, but are active participants in their own defense. They express TLRs<sup>128,129</sup>. They produce antimicrobial peptides and antiviral cytokines,



such as type I IFNs. They produce 'eat me' signals to bring in scavenger cells, alarm signals to activate local APCs, class-influencing signals to modulate local immune responses and chemokines to recruit cells for repair, remodelling and immunity. Finally, they may potentially also transmit 'health' signals to send away all of these cells when they are no longer needed. To fully understand these complex interactions we will need to step back, have another look, start using assays that measure a wider array of immune functions, and embrace the complexity that we find.

Polly Matzinger and Tirumalai Kamala are at the Ghost Lab, Laboratory of Cellular and Molecular Immunology, T-Cell Tolerance and Memory Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892, USA.

Correspondence to P.M.  
e-mail: [pcm@helix.nih.gov](mailto:pcm@helix.nih.gov)

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#### Competing interests statement

The authors declare no competing financial interests.