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Special Review

Defending and integrating an organism by the immune system

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Abstract

The Integrity model proposes that the adaptive immune response defends, protects and keeps vigilance over the unity of an organism. These functions conceptually rely on three signals that can explain them. All signals have a dual character. The signal-1 is the recognition of antigen or peptide/MHC ligand. The signal-2 comprises either help and costimulation or suppression and coinhibition. Lastly, the signal-3 signals tissues' *condition*, *state or integrity*. A part overlaps with the Danger-associated molecular patterns, and the other part should be detected by putative cell-surface molecules, intracellular factors or epigenetic events. They are called *the Integrity-associated molecular patterns* (IAMPs). The IAMPs originate from damaged (positive signal-3) or undamaged (negative signal-3) tissues. The positive signal-3 would induce costimulatory signal-2, whereas the negative signal-3 would induce coinhibitory signal-2 in APCs. However, in analogue reality, we might more likely encounter a range of signals supposedly sensed by a group of responder cells and integrated overtime (quorum sensing). The predominant option would sway the decision of the immune system to perform either defence or protection (active tolerance). Thus, the quorum sensing supposedly delivers two qualitative thresholds for T (and B) cells' decisions to defend or suppress. If these were not attained, the vigilance (anergy) of adaptive immunocytes for T-dependent antigens would ensue. These functions provide defence against pathogens and preservation of unity/integrity of an organism, which in turn permits protection of commensals.

1 | INTRODUCTION

Can we answer what life is by quantifying what it does? Probably yes, because life can be detected by the products of processes such as morphogenesis, replication, photosynthesis, carbon and nitrogen fixation, as well as chiral enrichment.[1-3] Any form of biology anywhere in the universe will code for life's information in complex collections of molecules that can very rarely form randomly and should be measurably different from lifeless material. The Assembly theory proposes that it can distinguish life-related residues in the extraterrestrial

matter by experimentally determining the complexity of molecules.[4,5]

Reversing the question, would quantifying the complexities of various life forms provide the answer to what they do? In other words, would assigning complexity rank to biological systems provide us with their function? Even if all data were available, measuring complexity expressed by numbers would be pointless. Hence, to explain complex systems, we need models of function.

Why did evolution select ever more complex life forms? Is there a factor or force we might have missed that drives development of more complex life forms? Or is it all just chance and necessity that takes life on an unended quest to achieve eternity?[6,7] We assume that all known life forms on Earth have descended from a common forerunner by Darwinian evolution. Unity and diversity are hallmarks of natural selection.[8] Perhaps then, unity is a drive for assembling life in more complex forms. A symbiosis (or commensalism) ensues if two life forms can unify. On the other hand, diversification drives speciation, increasing the chance for survival of 'life' per se. Unity plays a significant role in a biological system we studied for over a century – the immune system. In the integrity model, "unity" is the foundation for driving the diversity of the immune system, in phylogeny. However, as a caretaker of unity, immunity has a problem: what to put in and what to leave out? I want to discuss possible answers to this issue with this article.

The immune system can be divided into innate and adaptive. Let us briefly review what we know about it because I wish to suggest a possible explanation of its action based on the updated Integrity model.[9]

2 | INNATE LYMPHOID CELLS

Innate lymphoid cells (ILCs) are immune cells that lack antigen receptors, distributed during the early stages of development to reside in non-lymphoid tissues.[10,11] ILCs are thought to be involved in tissue development and remodelling, apart from immunity. We can distinguish five groups of ILCs (Figure 1) based on cytokines produced and transcription regulators involved in their generation and function: natural killer (NK) cells, ILC1, ILC2, ILC3 (which include lymphoid tissue inducer, LTi cells), and ILCreg.[12-17] All ILCs originated from early innate lymphoid precursors (EILP), some of which can differentiate into common helper innate lymphoid precursors (CHILP).[12]

Similarities between innate and adaptive immune systems:

1. The interesting feature of ILCs is that they resemble CD4 helper (Th) or CD8 cytotoxic T cell (CTL) responses of adaptive immunity.

2. The ILC1 type produces IFN-γ and has T-bet transcription factor such as Th1 subset cells, whereas ILC2 produces IL-5, IL-13 and shares GATA-3 transcription factor expression with the Th2 type (Figure 1). The ILC3 group corresponds to the Th17 type as they both express the RORγt transcription factor (the ILC3 can be further subdivided into CCR6 positive and CCR6 negative cells). Innate lymphoid regulatory (or ILCreg) cells have Id3 transcription factor expression, production of IL-10 (Figure 1) and show suppression of activation of other innate lymphoid cells such as ILC1 and ILC3.[18] They resemble various T regulatory (Treg) types, such as Tr1 and Tregs, which can inhibit adaptive immune responses of other adaptive immune subsets such as Th1, Th2 and Th17 types. Then, innate immunity's conventional NK cells originate from EILPs by acquiring transcription factors T-bet and Eomes that lead them to a phenotype similar to adaptive immunity's CD8 cytotoxic T cells (Figure 1) because they both show cytotoxicity, produce and secrete cytokines such as IFN-γ, TNF, GM-CSF and others [reviewed in references [11,12,17]].

3. The ILCs are known to change their subtypes (or shape-shift their phenotype), as illustrated by ILC2 and ILC3 trans-differentiating into other ILC types. So, one can identify ex-ILC2 cells that produce IFN-γ and function as ILC1. Similarly, one can demonstrate ex-ILC2 cells that produce IL-17A resembling the ILC3 group. Finally, there is an ex-RORγt/ILC3/ILC1 group expressing T-bet nuclear factor, which shifted phenotype into the one showing attributes of cytotoxic cells by secreting IFN-γ and TNF cytokines and producing perforin, granzyme and natural cytotoxicity receptors similar to ILC1 and NK cells.[12] In parallel to this, I suggest that similar trans-differentiations can also occur in adaptive Th types.

4. ILCs share features with other sessile lymphocytes – unconventional (innate) T cells, including invariant NKT cells (iNKT), mucosal-associated invariant T cells (MAIT), γδ T cells, intestinal intraepithelial lymphocytes (CD8αα⁺IELs) and tissue-resident memory T (T$_{RM}$) cells.[13]

Beyond these similarities, there are unique molecules (shared with non-immunity-related tissues) found in some ILC types: ILC2 produce novel molecular effectors such as amphiregulin, which mediates tissue repair,[19] and methionine-enkephalin,[20] which induces beiging of adipocytes from white adipose tissue (and limit obesity).

Innate stimuli that can activate ILCs are multiple consisting of predominantly soluble factors. For example, ILC2 cells receive activation from signals via IL-33 and TSLP from epithelial cells and myeloid cells, and IL-25
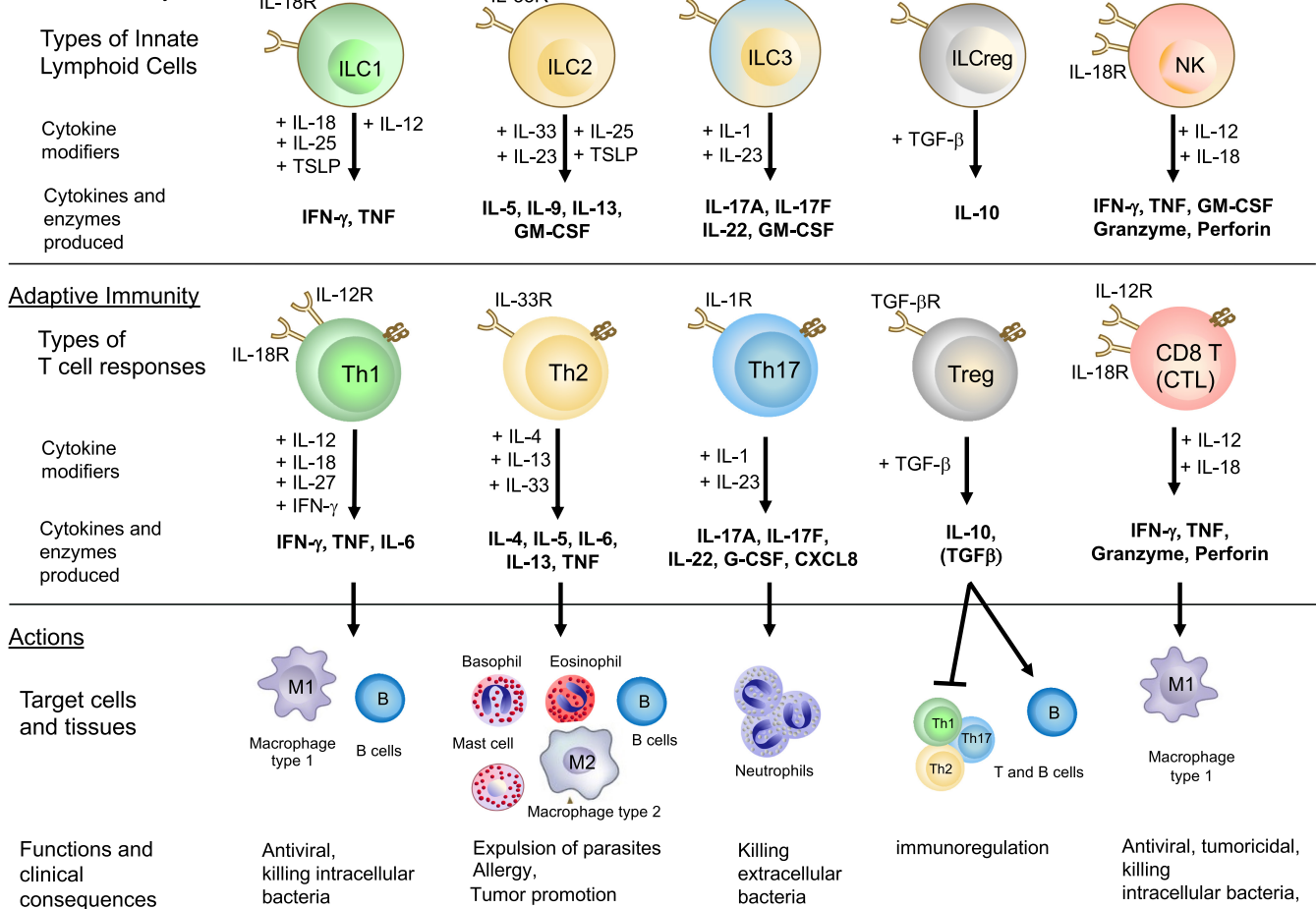
Innate Immunity

Types of Innate Lymphoid Cells

	IL-18R ILC1	IL-33R ILC2	IL-1R ILC3	TGF-βR ILCreg	IL-12R NK IL-18R
Cytokine modifiers	+ IL-18 + IL-25 + TSLP | + IL-12	+ IL-33 + IL-23 | + IL-25 + TSLP	+ IL-1 + IL-23	+ TGF-β	+ IL-12 + IL-18
Cytokines and enzymes produced	**IFN-γ, TNF**	**IL-5, IL-9, IL-13, GM-CSF**	**IL-17A, IL-17F IL-22, GM-CSF**	**IL-10**	**IFN-γ, TNF, GM-CSF Granzyme, Perforin**

Adaptive Immunity

Types of T cell responses

	IL-12R Th1 IL-18R	IL-33R Th2	IL-1R Th17	TGF-βR Treg	IL-12R CD8 T (CTL) IL-18R
Cytokine modifiers	+ IL-12 + IL-18 + IL-27 + IFN-γ	+ IL-4 + IL-13 + IL-33	+ IL-1 + IL-23	+ TGF-β	+ IL-12 + IL-18
Cytokines and enzymes produced	**IFN-γ, TNF, IL-6**	**IL-4, IL-5, IL-6, IL-13, TNF**	**IL-17A, IL-17F, IL-22, G-CSF, CXCL8**	**IL-10, (TGFβ)**	**IFN-γ, TNF, Granzyme, Perforin**

Actions

Target cells and tissues

	M1 Macrophage type 1 / B B cells	Basophil Eosinophil Mast cell / B B cells M2 Macrophage type 2	Neutrophils	Th1 Th17 Th2 / B T and B cells	M1 Macrophage type 1
Functions and clinical consequences	Antiviral, killing intracellular bacteria	Expulsion of parasites Allergy, Tumor promotion	Killing extracellular bacteria	immunoregulation	Antiviral, tumoricidal, killing intracellular bacteria,

FIGURE 1 Observed innate and adaptive immune responses (a simplified view)

from tuft cells. Also, IL-1α and IL-1β, derived from myeloid and epithelial cells together with IL-23, can activate ILC3 (Figure 1). The actions of ILCs include the production and secretion of soluble mediators. IL-18 can stimulate ILC1 to secrete IFN-γ and TNF after activation by myeloid-cell-derived IL-12. Likewise, IL-1 can stimulate ILC3s to produce IL-17, IL-22 and GM-CSF. Furthermore, IL-33 can induce ILC2 to secrete IL-5, IL-9 and IL-13 (Figure 1).

Thus, the IL-1, IL-12, IL-23, IL-25, IL-33 and TSLP affect ILC1, ILC2, ILC3 and NK cells in a specific way.[21] However, these innate stimuli are necessary signals for their activation but might not be sufficient. ILCs may require a stimulus via pattern recognition receptors such as the TLR or RIG to become fully active.

While circulating adaptive lymphocytes (B and T cells) and conventional NK replenish their numbers from hematogenous sources, the resident ILCs populate their niches early in ontogeny and remain in their environment throughout life. Their renewal depends on the support of local tissue-resident progenitor cells.[22] The markers on ILC include integrin α4β7 (that binds to MadCAM-1, which is expressed on high endothelial venules of MALT such as Peyer's patches) and the chemokine receptor CXCR6 that

guides them to the intestine.[23,24] Additional chemokines involved in intestinal residence are CCR9 receptors and CCR6, guiding ILCs to mesenteric lymph nodes.

3 | INNATE B AND T CELLS

In addition to ILCs, innate B and T cells carrying BCR and TCRs, respectively, were also described. Innate B cells include B1 (B1a and B1b) cells, marginal-zone B cells (derived from B-2 via transitional B cells) and some newly identified B cell subsets. Innate B cells have a limited diversity of germline-encoded BCRs that could be activated upon encountering innate stimuli. Similarly, innate T cells have a limited diversity repertoire (with their germ-line-encoded TCR) and seem to recognize non-classical MHC class I or MHC I-like molecules with lipids or self-derived peptides. Innate T cells comprise γδ T cells, CD1-restricted natural killer T (NKT) cells, mucosal-associated invariant T cells (MAIT) and intestinal intraepithelial lymphocytes (CD8αα+IELs).[13,25-28]

It seems that the primary (i.e. necessary and sufficient) signal for the ILCs' activation requires a pattern

recognition receptor such as the TLR or RIG. As there are no qualitative differences in chemical composition between self-tissue antigens and molecules derived from micro-organisms, the 'patterns' recognized describe quantitatively different molecular features frequently encountered in viruses, bacteria or parasites. In addition, stress/damage/integrity-related molecular patterns are present in the local tissue and circulation after tissue destruction and cell death by necrosis. Innate lymphocytes target self-antigens associated with the damage,[27] and it was proposed that the immune system recognizes a loss of tissue integrity after various forms of injury and reacts to it to re-establish homeostasis.[29-33]

On the other hand, marginal-zone B (MZB)-cell response is largely directed against thymus-independent antigens. They represent the front-line of the internal milieu in defence against micro-organisms that penetrated our organism and entered circulation. MZB cells in humans reside in the spleen between the lymphoid tissue of the white pulp and the circulation (red pulp). Outside the spleen, they are found in the inner wall of the subcapsular sinus of lymph nodes, the epithelium of tonsillar crypts and the subepithelial area of mucosa-associated lymphoid tissues, including intestinal Peyer's patches.[34] MZB cells are mixed with macrophages, dendritic cells and granulocytes in a web of stromal reticular cells in these areas.

Furthermore, induction of IgM secretion by MZB cell-derived plasmablasts seems to be dependent on splenic neutrophil B-helper cells (N_{BH}) that are different from the circulating conventional neutrophils (Nc). The N_{BH} activity also included contact-induced suppression of the proliferation of CD4 T cells activated via TCR and IL-2, which supports a view that N_{BH} cells are B-helper cells for immunoglobulin responses in a thymus-independent pathway.[34] Moreover, there are N_{BH1} and N_{BH2} subsets of neutrophils. The latter express markers similar to professional antigen-presenting cells involved in adaptive Th1 and Th2 responses, respectively. MZB cells produce MHC class II molecules, costimulatory CD86, B cell attracting chemokines CXCL12 and CXCL13, pattern recognition receptors TLR7, TLR8, and transcribe mRNAs for cytokines IL-1β, IL-6, IL-8, IL-12 and TNF.[35-38] The inhibitory molecules detected were cytokine IL-10 and its receptor (IL-10R), together with other immunosuppressive molecules such as CD11b, CD24, arginase, iNOS, IDO, SOCS1, secretory leukocyte protease inhibitor and progranulin.[35,39-42]

Another innate B cell subset recently described, natural killer B (NKB) cells, mainly reside in the spleen and mesenteric lymph nodes, have two markers, CD19 and NK1.1, and are proposed to be distinct from conventional B cells. NKB cells can produce large amounts of IL-18 (and IL-12) at an early phase of infection that could, in turn, activate ILC1s and NK cells.[43]

4 | ADAPTIVE IMMUNE CELLS (T AND B CELLS)

The similarities between ILCs and adaptive T lymphocytes are shown in Figure 1. When stimulated with cytokines such as IL-1, IL-18 and IL-33, during the activation via cognate peptide/MHC ligand recognition by TCR, the adaptive immune cells Th1, Th2, Th17, Tγδ17, and CD8 T cells produce and secrete similar cytokines to the ILCs. Th1 type produces cytokines such as IFN-γ, TNF, IL-6, whereas Th2 secrete IL-4, IL-5, IL-6, IL-13, GM-CSF. Further, Th17 (and Tγδ17) type has a characteristic secretion profile of IL-17A/F, IL-22, GM-CSF and chemokine CXCL8 (IL-8). Lastly, CD8 T cells produce IFN-γ, TNF cytokines, synthesize granzyme and perforin for their cytotoxic activity and appear similar in profile as their innate – NK cell counterparts.

These cytokines influence macrophages (M1 and M2), neutrophils, mast cells, and other granulocytes (eosinophils and basophils) to fight against viruses, tumours, parasites and intra- or extracellular bacteria. Besides, some of these actions may also provoke allergic reactions or promote tumour growth (Figure 1).

During T cell activation, IL-1 promotes the development of the Th17 phenotype of helper T cells, accompanied by IL-21, IL-23, and other polarizing cytokines such as IL-6 and TGF-β. In contrast, IFN-γ, IL-2, IL-4, IL-12 and IL-27 counteract the Th17 development.

The essential distinctions between innate and adaptive immunocytes are:

1. *BCR/TCR*. B or T lymphocytes express cell-surface B cell antigen receptor (BCR, Ig) or T cell receptor (TCR), respectively. Both receptors are highly variable glycoproteins, clonally distributed and unique for each individual of a species of higher vertebrates because DNA rearrangements generate them during ontogeny. B cell receptor interacts with a ligand differently than TCR. It has a membrane form (BCR) and a soluble form (Ig) that can bind to surface areas on large molecules in other cells' membranes or an aqueous solution, including proteins and polysaccharides. The T cell receptor has only a membrane form; one type can bind to antigenic-peptide/MHC complex and another to lipid/non-conventional-MHC complex on another cell's surface (antigen-presenting cell; APC)(reviewed in[44]).

2. *BCR/TCR accessory cell-surface molecules*. Adaptive immunocytes have species-specific cell-surface molecules that assist the BCR and TCR in their functions. For example, cell-surface molecules CD4 and CD8 represent species-specific transmembrane glycoproteins that can assist TCR in binding to respective

ligands, thereby increasing the avidity of interaction between T cell and APC. There are other molecules (including CD28, CTLA-4, PD1, CD40L, CD160, LAG-3, TIM-3, VISTA, BTLA, and CD244) that assist B or T cells in their function by upregulating or down-regulating actions performed by these populations following their activation. Effects of their actions can be measured, for example, by the production and expression of specific proteins either on cell surfaces of immunocytes or secreted in the environment. The examples are various classes of Igs and other effector molecules (reviewed in[44,45]).

3. *Intracellular factors and epigenetic modifiers.* Adaptive immunocytes have species-specific intracellular factors that can regulate various gene activities. These include nuclear transcription factors and epigenetic modifiers causing DNA methylation, histone modifications, microRNA (miR) production and *long non-coding* (lnc) RNA transcription. These can, in turn, regulate genes important for various activities, induce the cell cycle's machinery (proliferation), cause migration, homing and other effector functions, stimulate differentiation and trans-differentiation. Cytokines represent the largest group of necessary extracellular factors know to stimulate these modifiers.

How sufficient is our knowledge about the immune system? Although we gained much detailed information, we might have '*missed the forest by observing only trees*'. The concepts about the workings of the immune system are the Self-non-self discrimination (S-NS) theory,[46-48] the Pathogenicity pattern recognition (PPR),[49-51] the Danger,[52,53] the Calibration,[54-56] the Quorum,[57,58] the Morphostasis[59] and the Integrity[9,29,31] models. The Danger, the Morphostasis and the Integrity models are also known as 'alarmist', because they postulate an alarm signal needed for the immune system to become activated. Interestingly, there are exception-to-the-rule explanations for almost all these models in varying quantities. In trying to cover as many of such exceptions within a single concept (and without a need for adding an extra rule for each), some models (Danger, Calibration, and Integrity) deem the earliest (S-NS) as too complicated because there are too many exceptions. Recently, the Danger hypothesis has attained popularity in explaining immunity, as it unified many exceptions given by the S-NS (and PRR) models under a single concept. And lastly, I would argue that the Integrity hypothesis can unify even more exceptions, thus explaining more observations than the Danger model.

In attempting to resolve the suitability of these models, we should allow a scientific attitude to guide us to the next step of understanding: it consists of empirically supporting rules and predictions of a particular model, as well as having a willingness to revise or abandon its concept in the light of contradictory evidence.[60]

5 | THE IMMUNE SYSTEM, ACCORDING TO THE INTEGRITY HYPOTHESIS

Upon intrusion of a micro-organism, there are three main calls that the immune system must answer:

- A call to DEFEND an organism, using an active defensive response against the intruder.
- A call to PROTECT the unity/integrity of an organism, using suppression (active tolerance), for example, of autoreactive T cells (that escaped thymic negative selection). The process can also give protection (asylum) to commensals.
- A call to exercise VIGILANCE, using anergy. Provided an intruder appears to be non-harmful, the state of anergy ensues, which is passive tolerance. Vigilance is the readiness of adaptive lymphocytes to select one of the two calls mentioned above. Anergy could be also seen in lymphocytes that are self-reactive, but they were then supposedly activated under non-harmful circumstances (see later).

Defence (activation) and active tolerance (suppression) choices are thought to be dependent on signals by the cellular *decision-making assembly* (see later). After the decision, the determination of the class or type of the response follows. For thymus-dependent antigens, the defence is done by generating effectors such as T helper cell types Th1, Th2, Th17 and Th22, and the corresponding B cell classes of the response (IgM, IgG1, IgG2, IgG3, IgE and IgAs). For active tolerance, the decision would involve tTregs and pTregs (Foxp3Tregs, IL-35iTregs, Th3, and Tr), then Th2s with corresponding B cell class IgG4, innate lymphoid cells of regulatory type (ILCregs), and B cell regulatory cells (Bregs).

6 | DEFENCE

The invasion state compels a reaction from the host, and we historically refer to it as the immune response. According to the Integrity model, defensive action needs three signals to activate lymphocytes and APCs (Table 1).

Figure 2 schematically depicts the activation of T cells for defence against pathogenic 'parasitic microorganisms'

TABLE 1 The immune system's controlling (initiating) signals (the *Integrity* model)

The signals controlling the adaptive immune system by the Integrity model		
	Cells, processes and ligand – receptor combinations involved	
Signals	**T cells**	**B cells**
Signal-1		
(+)	Peptide/MHC; lipid/MHC; IAMs/MHC on DCs - TCR	Hapten; Antigen – BCR
(−)	As above – but resulting with tonic signaling by the TCR	As above – but resulting with tonic signaling by the BCR
Signal-2		
(+)	Costimulation (B7 on APCs); Th licensing of pCTL-CD28 on nT	Tfh help
(−)	Coinhibition (B7, B7-H1 on APCs); bystander suppression by Treg- CTLA4 on nT; PD1 on eT	Treg; Breg
Signal-3		
(+)	*pIAMPs*- pIAMP-R on T	*pIAMPs*- pIAMP-R on B
(−)	nIAMPs- nIAMP-R on T	nIAMPs -nIAMP-R on B.

Note: Signal-1(+) is a death signal without signals 2 and 3. Signal-1(-) is a negation of the death signal, and hence results with survival. Positive IAMPs overlap with DAMPs; negative IAMPs are not DAMPs, and represent anti- inflammatory ligands (for example, IL-1 bound to chromatin). Together with the signal-1 and signal-2, a balance between pIAMPs and nIAMPs is predicted to convey the signal-3, which is necessary and sufficient for the activation and active tolerance. All signals are represented as digital. In nature only analogue systems of molecular and cellular interactions exists, hence, a range of various signal-3s would be present at any time. Such signals are supposedly sensed by a group of responder cells and integrated over time (quorum sensing). This sensing weighs out the balance of pIAMPs and nIAMPs. Whichever option predominates, the result would sway the decision of the immune system to perform either defence or protection (active tolerance). Thus, the quorum sensing supposedly delivers two thresholds for the activation of T (and B) cells; one for each action. Failure in reaching any threshold, would cause a vigilant (anergic) state.

Abbreviations: eT, effector T cells; IAM, integrity-associated molecules; IAMP-R, IAMP receptors;IAMPs, Integrity-associated molecular patterns; nIAMPs, negative IAMPs; nT, naïve T cells; pIAMPs, positive IAMPs; Tfh, T follicular helper cells.

and viruses. In short, it starts with signal-3(+) deliverance to DCs in tissues. This signal is similar to the Danger model signal-0 (zero). The DCs cells upregulate costimulatory molecules - signal-2(+) - and migrate to the paracortex of the draining lymph node (reviewed in[31,33,44]). This signal has a counterpart in the Danger model which is called the signal-2.

In paracortex, the DCs can meet naïve T helper (nTh) and precursors of cytotoxic T cells (pCTL). The latter are activated by the recognition of the peptide/MHC ligand on DCs as signal-1(+), in combination with signal-2(+) and signal-3(+). Here, the difference with the Danger model becomes clear: the Danger model does not have the signal-3 involved at this stage. Activated T cells would proliferate, differentiate into effector cells and leave into tissues to exert their action. Some of the nTh cells will differentiate into T follicular helper (Tfh; expressing BCL6 nuclear factor) cells that would migrate into the cortex and help B cells to fulfil their activation and further differentiation.

T cell responses are not 'help' independent. They depend on many cells in their environment (which I call quorum sensing), not necessarily involving their own clonal descendant (I think it needs additional context, i.e. signals from stromal cells). Therefore, by stating here 'quorum sensing' mechanism, I have a different idea than already published 'quorum' hypotheses.[57,58] My quorum sensing idea stems from a postulated ability of eucaryotic cells to deliver some kind of 'decision' based on multiplicity of signals from surrounding tissue's well-being (immediate environment of the lymphocyte). Thus, there is no conceptual difference in the Integrity model between T and B cells' activation – only differences in cells and molecules involved.

Figure 3 shows the three signals-1, −2, and −3 (+) required for the activation of naïve or quiescent B cells during defensive action. Activated B cells would proliferate and differentiate into plasma cells, which migrate to tissues and secrete antibodies. Some B cells would remain in the follicle and then engage in somatic recombination combined with high-affinity maturation of their BCRs. The latter is a germinal-centre selection process that involves recognizing antigen complexed with a specific antibody (but perhaps with lower affinity), which on its other end (Fc portion) is bound to the Fc receptor of resident follicular dendritic cells (FDCs).
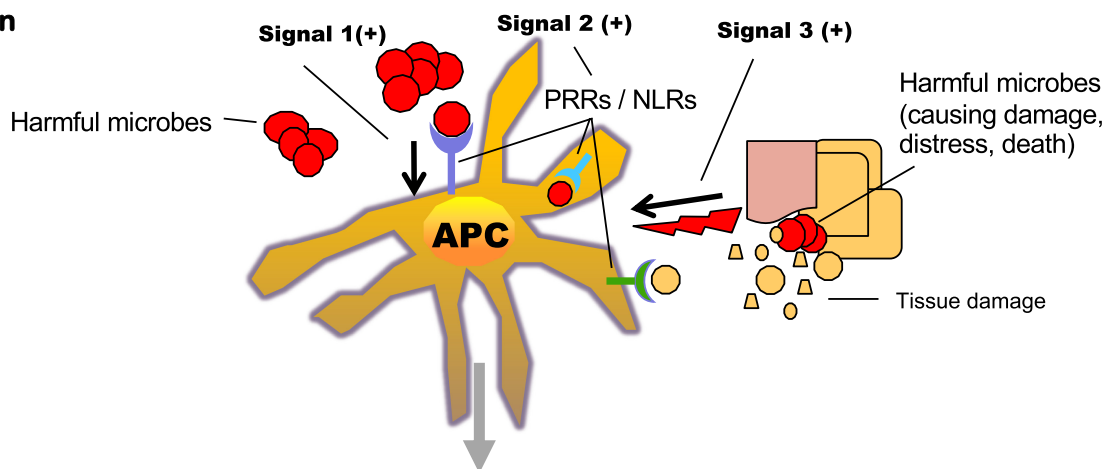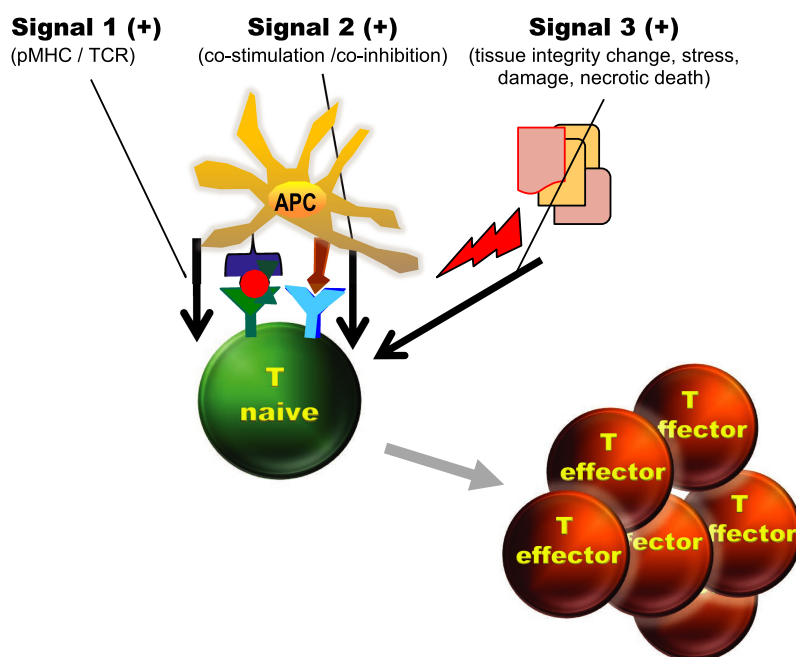
T-cell activation; The Integrity Model - 1

APC activation

T-cell activation

FIGURE 2 The Integrity hypothesis. The Integrity model employs three signals to explain actions of APCs and immunocytes. This is an example of the defence mode, when harmful microbes invade the host

6.1 | Signal-1: molecular ligand-receptor combinations

Signal-1 is conceived as a positive (+) or negative (-) stimulus that immunocytes internalize via transmembrane receptors. The Danger model does not have the signal-1 (-). In the Integrity model, positive signal-1 represents stimulation of naïve and quiescent B cells (via BCR), naïve or quiescent T cells (via TCR) and APCs (TLRs and other PRRs), causing activation and proliferation and further differentiation/maturation (together with other two signals). Negative signal-1 is exemplified with tonic signalling from the TCR, which can provide

survival signal for effector T cells (Table 1). It represents insufficient stimulation for proliferation, but perhaps opens a possibility for trans-differentiation. The choosing of 'negative' attribute in the signal-1 can be counterintuitive because it provides survival for adaptive T lymphocytes. Here is the rationale; per definition, signal-1(+) is a death signal in the absence of both, the signal-2 and the signal-3. This could be envisaged for example as a negative selection during thymic development. Theoretically, a negation (negative signal-1) of the negation (death) brings a positive effect (persistence), and hence an internalization of the 'negative signal-1' would lead to survival.
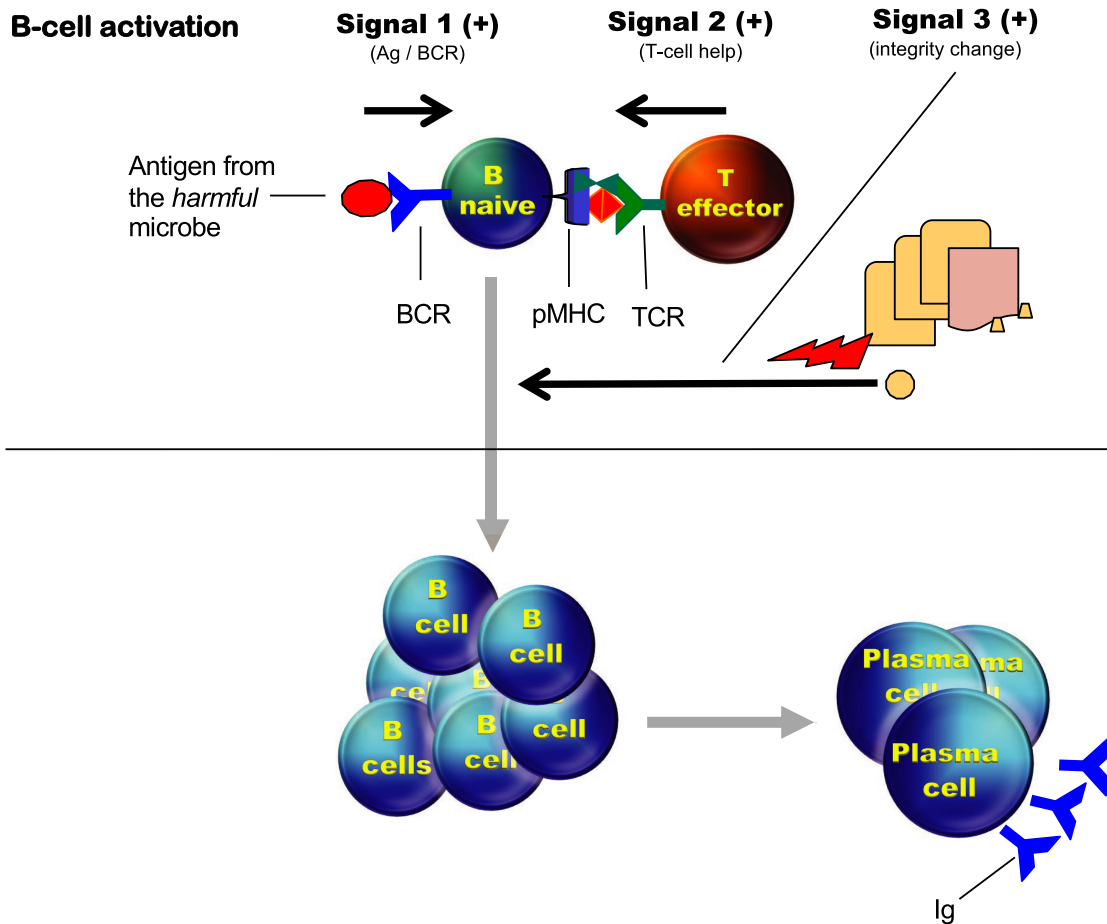
B-cell activation; The Integrity Model - 2

FIGURE 3 The Integrity hypothesis, part 2. This part shows the activation of B cells (for T-dependent antigens). This is a continuation of the defence mode shown in the Figure 1, when harmful microbes invaded the host and produced T cell effectors. The signal-3 transmits (in this case) the damage caused by harmful microbes. Ag: antigen, BCR: B cell receptor, pMHC: peptide-MHC ligand, TCR: T cell receptor, Ig: immunoglobulin

Major histocompatibility complex (MHC) molecules appear as sentinels or guardians having the possibility to deep-screen all encountered foreign antigens. In higher vertebrates, it has genetically expanded, probably for defensive purposes. All self-antigens can also be 'screened' (in form of short peptides) and presented by the MHC. A great number of these combinations serve to develop a T cell repertoire. There are particular self-antigen / MHC combinations in this repertoire that perhaps have an additional function. Namely, they might be selected to preserve the unity of an organism in evolutionary terms. In other words, MHC-restriction might be a remnant of the ancient unity/integrity drive.

Extensive studies suggest that the MHC-TCR binding has been evolutionarily selected.[61] Another important suggestion follows if we accept this conjecture: TCR-MHC interactions in the thymus must select high-affinity binding capacity; otherwise, lower affinity interactions would be lost in evolution. Evidence suggests that, for the generation of thymic Tregs (tTregs), there is a preferential selection of TCRs with high affinity to self-antigens.[62,63] I have argued that such high-affinity tTregs cells compete for binding to self-ligands on cortical epithelial cells and use this ability in peripheral active tolerance, thereby guarding the unity (integrity) of an organism.[32] Recent experimental evidence supports the competitive nature of tTreg generation.[64]

Furthermore, one kind of integrity signal exists in the form of self-peptides (or lipids) derived from household proteins of all tissues presented by the classical MHC or other sentinel molecules (such as non-classical MHC molecules) on APCs. These would be

integrity-associated molecules or IAMs (Table 1). The occurrence of household peptides/MHC complexes in local lymph nodes or spleen might indicate damaged tissue. The tTreg that meets this APC in the draining lymph node would detect it via its TCR (the signal-1). Thymic Tregs are produced by the positive selection of αβT cells that recognize self-peptide/MHC complex on cortical thymic epithelial cells with high-affinity-TCR. In the periphery, tTregs would suppress rare autoreactive T cell clones that escaped negative selection in the thymus. Some similarly sneaked-through autoreactive T cells might be activated during defence against a pathogen, mimicking self-antigen. Such clone could increase in frequency during infection and cause autoimmunity, provided their suppression failed by Tregs. Deactivation of such auto-destructive clone(s) would be tTregs prime function, as suggested previously.[32] In essence, they are poised to preserve the unity/integrity of an organism, being the last stand against autoimmunity.

The ground state of the immune system is affected by thymic regulatory (tTreg) cells, which suppress their neighbouring adaptive and innate cells by bystander inhibition (possibly docked on the same antigen-presenting cell).

6.2 | Signal-2: examples of processes, ligands, and receptors

Signal-2 represents the regulation of signal-1 and can also be positive or negative. Positive signal-2(+) is, for example, T cell help for naïve and quiescent B cells, CD4-T helper licensing of naïve and quiescent cytotoxic CD8 T cells, and costimulation of naïve and quiescent CD4 T helper cells (i.e. CD80, CD86) (reviewed in[31,33,44]). APCs can also receive the costimulatory signal via another type of PRR. For example, in DCs, if the signal-1 is received via TLR-9 in the suboptimal range, a suboptimal-range signal-2 via TLR-2 would co-stimulate the signal-1 leading to the activation and maturation of DCs (providing signal-3 is also present) (reviewed in[30]). Negative signal-2(-) would be provoking co-inhibition and exhaustion, like, for example, a change from CD28 to CTLA-4 expression on naïve T cells or upregulation of PD1 on effector T cells.

6.3 | Signal-3: molecular ligand-receptor combinations

The signal-3 stems from the recognition of *integrity-associated molecular patterns* (IAMPs) that can be detected by cells having pattern detection receptors such as

TLR, NLPs, RIGs, and yet unknown 'receptors' (collectively, we can call them IAMP-Rs). The IAMP-Rs would detect structures resulting from broken tissues, stressed cells, already destroyed (necrotic, apoptotic) cells, damaged intercellular matrix, or soluble molecules, including several cytokines (like IL-1α) alone or in complex with other molecules (like with chromatin).

For example, IL-1α has dual function.[65] It signals via surface membrane receptors, but it also translocates to the nucleus. If it is released from chromatin during necrosis or necroptosis,[66] it could promote sterile inflammation.[67] In most cases, necrosis is due to infectious agents, and thus, IL-1α will provide distress, damage and death signalling to adaptive immunity (the signal-3 +). However, if IL-1α stays bound to the chromatin – for an unknown reason, then it would not cause inflammation (as in the apoptotic cell death)[67] and is proposed to be of the signal-3(-) kind (Table 1), which is not a dangerous, but perhaps friendly signal.[53] Apoptotic death is physiologic during embryonal development, tissue regeneration and maintenance, thus avoiding the generation of autoreactivity.[68]

Integrity-associated molecular patterns (IAMPs) and their receptors (IAMP-R) are predicted to convey signal-3. Positive (p) IAMPs overlap with DAMPs; negative (n) IAMPs are not DAMPs and represent anti-inflammatory ligands (for example, IL-1 bound to chromatin). In nature, only analogue systems of molecular/cellular interactions exist, and hence, this digitized concept (like all other models) is a simplified explanation of such situations.

6.4 | The quorum of cells

According to the Integrity model, the immune system would function as a *decision-making* cellular assembly based on the communication between its constituents via *quorum* sensing of signals. The quorum sensing weighs out the balance between signals delivered by pIAMPs and nIAMPs, perhaps by integrating their influence overtime (Table 1). If it reaches a particular threshold for the activation (via the prevalence of pIAMPs), it would kick-start the defensive action. If, however, another threshold is reached (via the dominance of nIAMPs), an active tolerance choice would be provided for T or B cells. Thus, the decision to perform either defence or protection (active tolerance) lies in quorum sensing. Without attaining any of the two thresholds, immunocytes would remain in a vigilant (anergic) state (Table 1, Figure 4).

The assembly of immune regulator cells capable of quorum sensing and sending signal-3 to the adaptive immunocytes include DCs, conventional CD4 and CD8 T cells, tTregs, B cells, FDCs, innate lymphoid cells (ILC1, ILC2, and ILC3), monocytes, macrophages, NKs, and

stromal cells. In the paracortex of lymph nodes, stromal cells are usually referred to as fibroblastic reticular cells, and in the cortex, B-cell interacting reticular cells (for a review, see[69]). They can supposedly transfer information about the integrity of the local environment. For example, the information could involve the secretion of soluble IL-1α (*positive* signal-3) or its counterpart bound to chromatin (negative signal-3). This quorum-based decision of assembled immune-and stromal cells is the start of the defensive immune response (that would reject invading pathogen or parasite). After the response has gone its course, with the pathogen rejected and tissue healed, the information about the state of tissues would change in several weeks. The restored tissue integrity would be signalled to the LNs with *negative* signal-3. This event would be transmitted via a quorum-decision mechanism, and it would reverse the previous activation signal (diminish the signal-3 below the threshold for activation of defence). This quorum-sensed choice would downregulate costimulatory molecules on DCs and upregulate coinhibitory ones (i.e. negative signal-2), which would lead to Tregs' overpowering influence. For example, this could start a generation of conventional T cells into pTregs (perhaps, by conversion via tTregs), thus establishing negative feedback to ongoing immune response (Figure 4).

7 | SELF-PROTECTION AND ACTIVE TOLERANCE (OF NONSELF)

The function of the immune system is guided by two hallmarks: a need for the defence of an organism (immunity), which we discussed, and a need for an association of cells (unity; integrity). The latter allows commensalism between the host and an invading micro-organism. It is, essentially, a survival niche for a commensal that is protected by the active tolerance mechanism.

In evolutionary terms, the loss of potential commensalism might be equally disadvantageous as the threat of infection and death. These traits perhaps balance each other. It could be seen as to why the immune system necessities to integrate signal-3 and selects one course of action. If 'pros'(-) and 'cons'(+) for commensalism were integrated by the quorum sensing of responding cells, a 'result' would ensue in form of either tolerance (protection) or activation (defence). The quorum of responding cells would be the same as for the defensive action and include DCs, APCs, antigen-specific T and B cells, ILCs, NKs, monocytes, stromal cells and possibly other kinds of cells of the immune system. As the decisions are further controlled by the tissues where the effector immune cells are directed, I suggest we call this hallmark a '*histocracy*'.

Perhaps, the *histocratic* influence of tissues might be better illustrated with the following issues:

1. Both foreign and syngeneic grafts possess pIAMPs and yet syngeneic grafts are not rejected. As an explanation, I propose that despite pIAMPs presence in both cases, the tTregs specific for foreign graft are not selected during ontogeny, and hence, the foreign grafts would be rejected. The tTregs for syngeneic grafts are previously selected in the thymus, and consequently would hinder rejection.

2. Healed-in foreign grafts (i.e. lacking pIAMPs and presumably possessing nIAMPs) are rejected, even by an immune system that first develops in the presence of the healed-in graft (for example a RAG-/- mouse given a foreign tissue graft and months later reconstituted with host hematopoietic stem cells). The answer to this issue is that host hematopoietic stem cells cannot generate tTregs in the thymus specific for the healed-in foreign graft. This is because the tTregs are selected only for self-peptide/self-MHC combinations, and not for foreign-graft-peptide/self-MHC ones. The lack of the foreign-healed-in-graft specific tTregs would tip the balance towards rejection of the healed-in foreign grafts in the periphery.

So, if tTregs protect tissues of an organism by recognizing particular tissue-specific peptide-MHC combinations by suppressing self-reactive immunocytes, how do they protect commensals? It seems that micro-organisms might evolutionarily seek a 'survival niche' by imitating the same self-integrity peptides and hence be protected. There is no preference in that – natural selection would eventually decide on the winner. Usually, those that can remain under the protection of tTregs, would not cause harm and inflammation, and in that, they are already beneficent (in commensalism), as they outcompete pathogens. However, some pathogens might eventually slip through the defence. Or, they could pretend to be non-harmful for a long time, and then suddenly switch to causing damage or disease. However, such pathogens might be very rare, as each sneaked pathogen (mimicking self-protected antigens) would be commensal for only a single individual and would not be beneficent in other hosts.

In evolutionary terms, the protection of a commensal with tTregs might be advantageous for a species because the threat of a pathogen doing the same is minimal (for the reason mentioned above). In other words, if a pathogen mutates in a way as to imitate that individual's tTregs-niche protection, it would only result in the demise of that particular individual, and the rest of the species would remain safe.

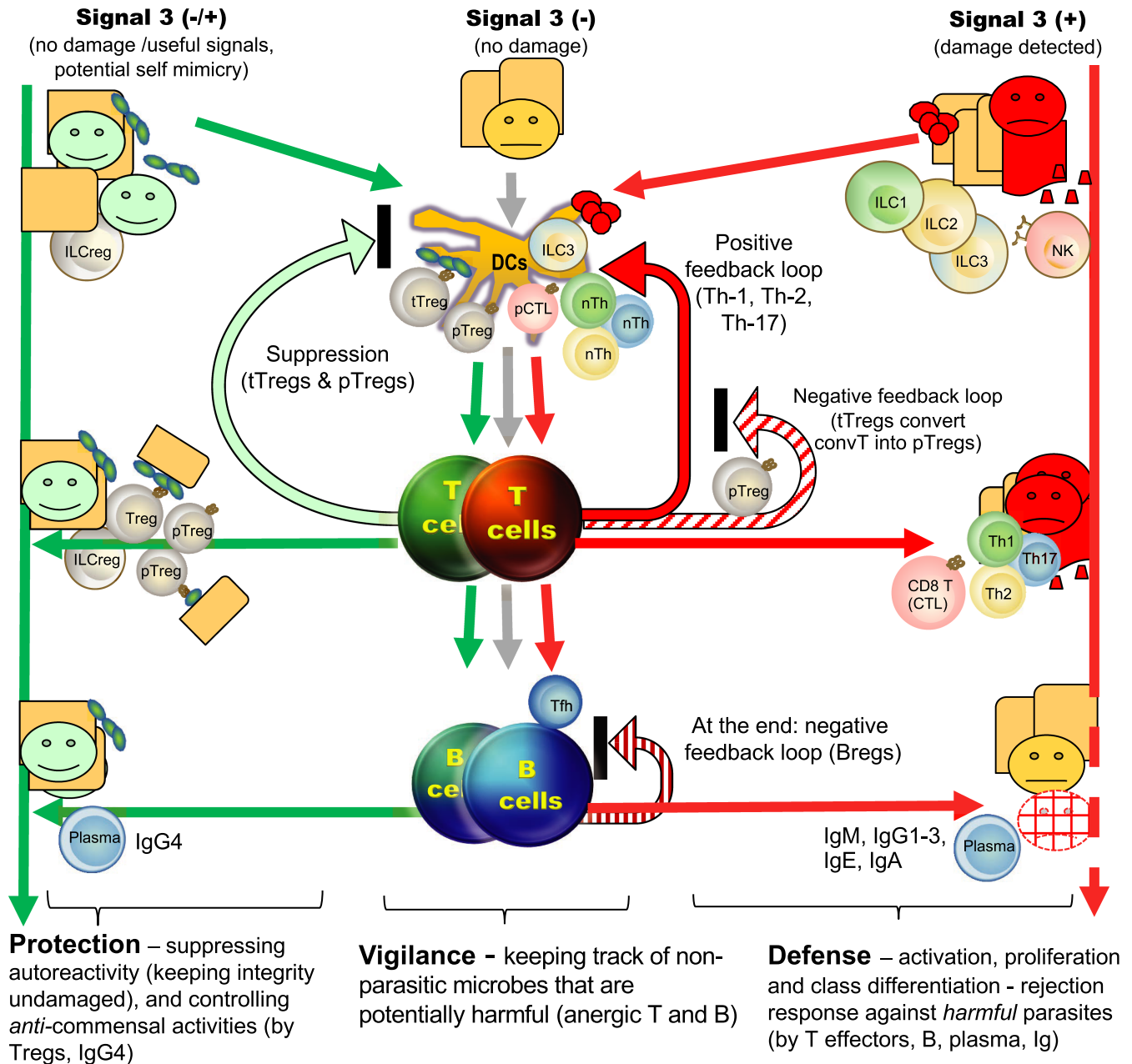## Proposed pathways and outcomes – the Integrity model

FIGURE 4 A schematic view of three outcomes (functions) of the adaptive immune system predicted by the Integrity model

In conclusion, the tTreg-protected niche would protect tissues from residual autoreactivity and protect commensals.

8 | VIGILANCE

The immune system has to be constantly on the alert and exercise vigilance because the mutual benefit of commensalism can abruptly end. It may be envisaged that a sudden change in environmental conditions would lead to competition for food, energy or other resources. This would surpass the gains from commensalism, and former partners might become enemies.

Tissues supposedly give information to the immune system (DCs-ILCs-NKs-NKT-Mϕ-T-B cells) via various signal-3. The innate immunity cells survey tissues looking for signs of *positive* IAMPs, while constantly reporting the *negative* signal-3 to tTregs. Vigilance is the readiness of anergized conventional T and B cells to choose defence or protection by quorum sensing of the signal-3. Consequently, it includes type-determination of T and class-switching of

B cells. In the former case, trans-differentiation might also occur, for example, when quorum sensing involves cytokines and other factors that could lead to type switching, such as Th1 into Th2, or Th17 into Th1 response.

The class determination decision is interspersed within the nature of other signals (especially signal-3).

9 | AUTOIMMUNITY AND CANCER

In Table 2, predictions are listed concerning normal homeostasis and infection, which we have just discussed. The autoimmune diseases and cancer are left to be described and explained with three functions predicted by the Integrity model.

Autoimmunity can arise when there is an error in a deletion process like negative selection during B and T cell development. The errors can be multiple, and one of the common ones is the loss of the self-peptide presentation (via the autoimmune regulators AIRE or FEZF2 that cause promiscuous peptide expression; reviewed in[70,71]) by thymic epithelial medullary cells leading to autoimmune polyendocrinopathy syndrome type 1 and other autoimmune syndromes.[72] Various autoimmune diseases may supposedly develop if tTregs fail to be generated in the thymus. Similarly, peripheral Tregs could have a failure in their generation. One of the culprits might be the loss of tTreg-solicited conversion of conventional T cells into pTregs.

Cancers' hallmark involving avoidance of immune cell attacks is a complex phenomenon.[73] It probably involves a range of abilities from imitation of normal undamaged integrity to mutations that lead to escape from immunosurveillance (Table 2). For example, it includes upregulation of signal-2(-) molecules such as B7-H1 and H2 (PD1-Ligands) for effector T cells. The effectors assume an 'exhausted' phenotype; in other words, they become anergic/tolerant of cancer cells. Similarly, it includes premature upregulation of inhibitory signal-2(-) molecules preventing (or reversing) early T cell activation in the draining lymph nodes (like CTLA-4). The CTLA-4 can bind the same ligands as the signal-2(+) molecule CD28, which are B7.1 and B7.2 (CD80, CD86) (reviewed in[73]).

How can tumour immunosurveillance take effect at all, if cancers have nIAMPs? Initial tumour tissue growth includes protection by tTregs because they possess nIAMPs. However, when cancer mutates nIAMPs they can become pIAMPs and thus immunosurveillance starts. Cancers with mutated nIAMPs (turned pIAMPs) are rejected by a similar mechanism as the healed-in foreign grafts (see issue 2 in section 7.2).

Furthermore, evidence suggests skewing of T helper responses in cancers such as NSCLC type into predominantly Th2 or Treg types.[74] The immunosurveillance is achieved by Th1 and CTLs specific for tumour neoantigens as tumour-peptide/MHC ligands. The skewing into Th2 type was suggested to be a mechanism of the immune escape of tumours.[75] I suggest that the skewing into any different type of responses would avoid immune cells' attack. The detailed mechanism of such class determination according to the Integrity model is planned to be published elsewhere.

10 | CONCLUDING REMARKS

Although the Integrity model might seem to have answered some issues better than others, there are still many unanswered questions. We still cannot predict how long the disease protection from vaccinations would last, even though more research on molecules involved in signal-2 and signal-3 might reveal some answers. Similarly, we cannot direct the immune system to attack and eradicate every cancer. Here, it would also help research more signal-1 related targets associated with cancer and, of course, signals-2 and −3 used by cancer to avoid immunosurveillance.

Another question is whether we could therapeutically control autoimmune diseases without completely switching off the immune system, thus rendering patients immunodeficient? We cannot predict which part of the self-destructive component of the immune system we should suppress in search of the cure. Perhaps, the research on signal-3 might hold the remaining clue.

Which molecules should we target therapeutically to have transplantation tolerance without switching off the immune system (for the same reason)? Can we use a similar strategy as nature has already done during pregnancy (in tolerizing foreign antigens of a foetus)? Here, the answers might also lie in the research of the signal-2 and −3. Some of these signals are related to Tregs. Their contribution and interactions are required to understand the underlying processes fully.

Finally, I suggest that the immune system might have a larger role in biology that previously anticipated. The role is dual and dates back to our earliest predecessor. We know two drives comprising unity and diversity that are the hallmarks of evolution.[8] And both could be found in the function of immunity. For example, the former drive could have selected cells to become an integrated multicellular organism and promoted the assembly of various cells to gain an advantage over competitors in accessing resources for survival. The other drive diversified immune responses. In short, our immune system contains the defence using a diversified repertoire of cellular functions and specificities, but it also has a remnant of the

TABLE 2 The immune system's hallmarks (protection, vigilance and defence) according to the *Integrity* model

Predictions in normal and diseased states

Functions of the immune system	Predictions in normal and diseased states			
	Homeostasis	Infection	Autoimmune disease	Cancer
1. Protection	Active tolerance by tTregs: suppression of autoreactive T-cell clones; protection of commensals (suppression of anti-commensal T cells)	Protection of self-tissues by suppression of rare autoreactive clones (by tTregs and pTregs). Asylum to commensals (Tregs)	Failure of correct Th type determination, or mistake in pTregs generation, or tTregs fail to convert conventional T into pTregs	Immunosurveillance via Th1 and CTL. Cancer avoids attack by Imitating normal integrity (*providing negative signal-3*), upregulating exhaustion markers, generating pTregs, and/or causing Th type shift
2. Vigilance	Reversible peripheral tolerance by anergic T/B: anti-self (i.e. soluble self-antigens) and anti-commensal antigens	Anergic T/B cells are generated, if microbes appear to be neutral (non-harmful)	Anergic autoreactive T/B cells become mistakenly activated	Cancer avoids immune cell attack by usurping vigilance. Cancer imitates neutrality, anergizing or shutting down immune cells' effectors
3. Defense	Potential to activate anti-nonself T/B-cells: their repertoire is clonally selected (in the thymus / bone marrow) to recognize nonself	Activation of anti-nonself T/B cell repertoires, with generation of effector cells. Killing (rejecting) *harmful* microbes and parasites (by T and B effectors or their products)	Mistake in tolerizing autoreactive T/B immunocytes	Cancer mutations lead to escape from immunosurveillance, and failure to activate T and B cells

Note: Predictions of what happens in the case of infection, autoimmunity, and cancer are listed. Under normal circumstances the immune system functions as a *cellular / soluble-mediator decision-making* organ based on communication between DCs, NK, M, T and B cells, perhaps *via quorum* signalling. This communication is required for host homeostasis.

ancient unifying force because it still keeps beneficent commensals in.

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