Clinical Biochemistry 49 (2016) 324–328

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Mechanisms of immunological tolerance

Herman Waldmann

Sir William Dunn School of Pathology, University of Oxford, United Kingdom

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 11 May 2015
Accepted 17 May 2015
Available online 30 May 2015

Keywords:
Tolerance
Regulation
Biomarkers

ABSTRACT

There is increasing interest in establishing diagnostic markers of immunological tolerance applicable to efforts to minimize drug immunosuppression in transplantation and chronic immunological diseases. It is hoped that an understanding of the diverse mechanisms that can contribute to tolerance will guide efforts to establish diagnostic tolerance biomarkers. Not only would these be valuable for management of autoimmune diseases, transplants and allergies, but they might also guide efforts to override tolerance processes in cancer and vaccine development. Where tolerance is generated by deletion or inactivation of antigen reactive lymphocytes, it is unlikely that any long-term-valid blood biomarkers might be found. Where tolerance is mediated by active regulatory mechanisms, indicators that can be usefully measured may emerge, but these would likely show significant heterogeneity reflecting the diversity of active tolerance processes operating in different individuals. Given this, the most useful "kits" might be those "smart" enough to detect this diversity of tolerance players.

1. Introduction

The immune system has evolved to protect against microbial pathogens. It uses an ancient defensive arm of "innate" cells and proteins both to provide signals of microbial danger, and as a first line of defence. The protection that innate immunity offers is not selective to individual microbes, and also lacks a memory for the inciting agent. In contrast, the more recently evolved adaptive arm makes use of a vast array of pre-committed receptors that are clonally distributed amongst lymphocytes capable of delivering memory. These receptors provide coverage to protect against the vast majority of the pathogens that might be encountered. The receptor repertoire, unique to each host, arises through a limited set of inherited gene segments undergoing further random rearrangements and somatic mutations. Lymphocytes use these receptors to recognize antigen and to deliver signals determining their fate, be that immunity or tolerance. An inevitable risk of such receptor diversification is the generation of receptors to "self" and consequent autoimmune disease. Autoimmunity is prevented by lymphocytes having to pass through many developmental checkpoints, as well as control through diverse failsafe systems. Receptor generating mechanisms have no way of predicting the "self" within which they develop. Consequently, self-tolerance must be an acquired process rather than one that is inherited, as elegantly shown by Medawar and his colleagues [1].

2. Mechanisms of tolerance

The self-tolerance processes are called into play from the time lymphocytes first express their surface-receptors for antigen. The availability of genetically modified mice [2–7] and monoclonal antibody probes [8] has provided some of the best tools for establishing mechanisms. Transgenic mice expressing a single type of antigen receptor on any given lymphocyte subset have been invaluable for tracking and defining the fate of antigen specific cells. Mutant mouse strains exhibiting spontaneous or easily-induced autoimmune disease have provided opportunities to study how tolerance can be broken. Experiments of nature such as APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) [9–11] and IPEX (immune dysregulation, polyendocrinopathy, enteropathy and X-linked inheritance) [12,13] have highlighted the importance of the thymus in ensuring tolerance in thymus-processed lymphocytes (T-cells). In addition, many genetic mutants have been identified where defects in distinct signalling pathways interfere with the generation of self-tolerance. The form of tolerance occurring in the primary lymphoid organs is often referred to as "central", whilst that occurring in lymphocytes once they have migrated out of these sites, is commonly referred to as "peripheral".

2.1. Central mechanisms

A large proportion of self-reactive T-cells and bursa/marrow-derived lymphocytes (B-cells) are purged in their corresponding primary lymphoid organs [4,14,15]. For T-cells this process requires that the T-cell receptor for antigen (TCR) has a defined affinity for antigen [16]. For ubiquitous self-antigens T-cells encounter processed fragments of those antigens on dendritic cells in conjunction with host MHC, as one might expect. However, the thymus is also able to inactivate T-cells reactive with antigens thought to be restricted to tissues remote from the thymus. This happens because many "tissue" restricted antigens are promiscuously expressed within the thymus by its medullary epithelial

E-mail address: herman.waldmann@path.ox.ac.uk.

http://dx.doi.org/10.1016/j.clinbiochem.2015.05.019
0009-9120/

cells [10,17], under the influence of the AIRE (Autoimmune Regulator) gene. Mutations in AIRE give rise to the clinical APECED syndrome, characterized by widespread autoimmune disease in man [9,18] and mouse [17]. In addition, the thymus also serves as a site of development of a subpopulation of CD4 T-cells with the ability to damp any autoreactive T-cells that escaped deletion. This subpopulation of T-cells which express the forkhead transcription factor FoxP3 are commonly referred to as natural (n) or thymic (t) regulatory T-cells (Treg). The IPEX syndrome is caused by mutations in the FoxP3 gene.

In a similar vein B-cells are also purged of self-reactive cells in the bone marrow [19]. Multivalent presentation of antigens is more effective for B-cell deletion than monomers. B-cells may also express alternative receptors rather than die, a result of further genetic rearrangements known as receptor editing [20].

2.2. Peripheral mechanisms

The primary lymphoid organs contribute a substantial amount towards self-tolerance. There are, however, additional checkpoint mechanisms and failsafes that operate in the periphery.

2.3. The role of lymphocyte cooperation and co-stimulation in determining tolerance or immunity

Cooperation between antigen-specific T-cells and other lymphocytes has long been recognized as a necessary component of immune responses [21,22]. Where such cooperation is unavailable, or inhibited, tolerance may be a consequence. For the few rogue self-reactive T-cells emerging from the thymus, cooperative partners would only be available at a non-useable low frequency, and so tolerance would be the more likely outcome [3,23,24]. This may explain the necessity for "linked-recognition" in humoral antibody responses [25,26] where T-helper cells recognize distinct epitopes from the identical antigen that their partner B-cells bind. B-cells internalize the antigen and process antigen for presentation on the surface membrane MHC Class II. T-helper cells recognizing that peptide–MHC complex would then engage and cooperate with such B-cells. In the absence of T-cell "help" the unprocessed antigen can provide a signal that can alone lead to B-cell tolerance. Cooperation between T-cells does not require that they need to come together physically. Rather, one (helper) partner can license (activate) dendritic cells, so that they are empowered to stimulate the other partner when it eventually engages that DC [27]. Such "licensing" by helper T-cells involves delivery of signals through costimulatory ligands such as CD40L and CD28 to corresponding receptors (CD40 and CD80/CD86) on dendritic cells. As a consequence the DC upregulates many immune stimulatory molecules [28,29], that have the potential to facilitate signalling of (helping) other T-cells that the DC later engages. This property of DC puts them in a pivotal position to be modulated by cell products generated in their local microenvironment, not least of which are regulatory T-cells. It also puts them at risk of being inappropriately licensed by microbial and other activating stimuli, so risking autoimmune disease [30]. From a therapeutic standpoint, the prevention of "licensing" with probes that block T-cell–APC interactions will favour tolerance [31–34].

2.4. Tolerogenic dendritic cells

Given the important part that DC "licensing" plays in driving immunity, it is clear that events that "decommission" DC would favour tolerance. DC which are immature or whose functions are down-regulated by regulatory T-cells or inhibitory cytokines are consequently capable of inactivating T-cells, and indeed to direct some CD4 T-cells towards regulatory function [35–38].

2.5. Co-inhibitory molecules and their contribution to tolerance

Not only do cells of the immune system display ligands capable of activating interacting cells. They also exhibit others capable of providing damping signals. The classic example is cytotoxic T-lymphocyte protein (CTLA4) [39], which may operate by stripping co-stimulatory receptors off the surface of DC [40]. Others have increasingly come to prominence in recent times, especially as targets for checkpoint blockade in cancer immunotherapy. These include programmed cell death receptor ligand PDL1 [41,42], T-cell immunoglobulin mucin family (TIM) members [43], B-cell inhibitory (ITIM) receptors [44,45] such as FcRII, and members of sialic acid binding Ig-like lectins (Siglecs) [46] and many others [47]. Inhibitory Siglecs on B-cells [48] have been suggested to damp B-cell responses to self-proteins which may be decorated with sialic acid residues in a way that microbes are not [49].

2.6. Regulatory cells

Despite much early scepticism it has become clear that the immune system exploits a number of different cells to inhibit or regulate immune functions. These range from subsets of CD4 T-cells, now known to express the transcription factor FoxP3 [50–54], others that secrete IL-10 [55,56], subsets of B-cells [57,58], and even subsets of hemopoietic stromal cells [59,60]. The best studied of these are the FoxP3+ regulatory cells. These characteristically, but not uniquely express the α-chain of the IL2-R (CD25), for which IL-2 provides a growth enhancing stimulus [61]. As mentioned earlier, studies in infants with IPEX syndrome, and the mutant scurfy mouse exhibiting widespread autoimmune disease, established forkhead transcription factor 3 (FoxP3) as the key element determining the development of these CD4+ regulatory T-cells. Surrogate surface markers knocked in to the Foxp3 locus have allowed isolation and ablation of these cells, and implicated them in ensuring self-tolerance [62], and many forms of "therapeutic" tolerance [63].

The majority of FoxP3+ Treg develop in the thymus and are therefore referred to as tTreg. These are thought to be self-reactive possessing a different T-cell receptor repertoire to conventional peripheral CD4+ T-cells. Another set of FOXP3+ Treg develop from naive CD4+ T-cells in the periphery, and are referred to as pTreg. Naïve CD4+T cells can be induced to express FoxP3 in vitro by providing antigen in the context of TGFβ and mTOR inhibition [64–68]. In vitro induced pTreg can be shown to prevent T-cell-mediated tissue damage in-vivo [69,70], but their physiological role in self-tolerance is currently unclear.

Whatever the source of Treg, it seems that their commitment to that lineage requires that they undergo certain epigenetic changes [71]. Foxp3+ T-cells without such changes might only be exhibiting their regulatory functions transiently.

2.7. Targeting regulatory T-cells for therapeutic purposes

Even before much was known of tolerance mechanisms immunologists have attempted to exploit the little they knew in attempts to achieve tolerance to foreign proteins, and in allergy, autoimmunity and transplantation. The discovery of monoclonal antibodies provided researchers with abundant new probes for this purpose. Based on the notion that interference with T-cell help might allow tolerance as a default response, short pulse treatment with monoclonal antibodies to T-cell coreceptors (anti-CD4 and anti-CD8) was shown able to induce tolerance to foreign proteins and to allografts [31,34,72]. Tolerance was found to depend on regulation by CD4 T-cells, and could be transmitted through multiple serial transfers of splenocytes into sequential recipient mice. Ablative studies established that the cells responsible were FoxP3+ Treg and, in part, the pTreg among them [63]. Tolerance was shown to be long lasting whereby the first cohorts of Treg enabled further antigen-specific Treg to be recruited into a process known as "infectious tolerance" [53]. Persisting antigen in the host was shown to