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Review

History and mechanisms of oral tolerance

Rafael M. Rezende, Howard L. Weiner*

Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, 60 Fenwood Road, Boston-MA, 02115, USA

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ABSTRACT

Since its first description by Wells and Osbourne in 1911, oral tolerance has intrigued researchers due to its potential for therapeutic applications. Oral tolerance can be defined as an inhibition of specific immune responsiveness to subsequent parenteral injections of proteins to which an individual or animal has been previously exposed via the oral route. Tolerance induction to commensal bacteria and dietary proteins represents the major immunological event taking place in the gut in physiological conditions. Multiple mechanisms have been proposed to explain the immune hyporesponsiveness to fed antigens: low doses of orally administered antigen are reported to favor active suppression with the generation of regulatory cells, whereas high doses would favor clonal anergy/deletion. In this review, we highlight historical aspects and the mechanisms proposed for oral tolerance induction.

1. Introduction

In 1953, Peter Medawar coined the term "tolerance" [1]. It was then incorporated by Burnet [2] in his classical Clonal Selection Theory. Burnet's concept of tolerance involves three basic assumptions, where (i) the primary function of the immune system is to protect the organism from pathogens or from non-self materials; (ii) to perform this function, the immunologic response must be an inflammatory response; and (iii) tolerance is a negative counterpart of the immune system due to neonatal deletion of "forbidden clones." Thus, oral tolerance has classically been defined as the specific suppression of cellular and/or humoral immune responses to an antigen by prior administration of the antigen by the oral route [3]. It presumably evolved as an analog of self-tolerance to prevent hypersensitivity reactions to food proteins and bacterial antigens present in the mucosal microbiota.

Oral tolerance is of paramount importance because it contributes to the balance between exogenous antigens that come from diet and commensal bacteria, and the self-components of the body at the mucosal surface. The mucosa of the small intestine alone is estimated to be 300 m^2 in humans and there are 10^{12} lymphoid cells per meter of human intestine [4]. Approximately 130–190 g of dietary proteins is absorbed daily in the gut [5] and the number of bacteria colonizing the colonic mucosa can reach 10^{12} microorganisms/g of stool [6]. These antigens are crucial for the maturation of the immune system in the post-weaning period and oral tolerance rather than inflammatory immune response is established in order to keep homeostasis. Thus, oral tolerance is a form of peripheral tolerance that evolved to treat external agents that gain access to the body via a natural route as internal components that then become part of self. In this review, historical aspects of oral tolerance as well as the mechanisms involved in its induction are highlighted.

2. Historical aspects

2.1. Immunological tolerance

"Immunological tolerance" has often been defined as a mechanism by which the immune system prevents pathologic auto-reactivity against self and thus prevents autoimmune diseases. A classical study published in Nature in 1953 by Billingham, Brent, and Medawar demonstrated that immune responses against a defined set of antigens could be abolished, or at least attenuated, using a biological approach [1]. This work was inspired by the study of Ray Owen [7], who for the first time observed the phenomenon of immunological tolerance in vivo by showing the coexistence of two types of erythrocytes in the blood of dizygotic cattle twins: each calf contained a proportion of red blood cells belonging genetically to itself, mixed with red blood cells belonging to the zygote lineage of its twin. For different proposes, Billingham and Medawar were investigating the fate of skin allografts in young cattle in order to develop a test to distinguish between fraternal and identical twins. Surprisingly, they found that skin grafts transplanted from one twin to the other were accepted, irrespective of the origin of the twins [8]. Next, to further prove that tolerance to a known antigen could be induced in vivo, they demonstrated, by inoculating *in*

* Corresponding author.
E-mail address: hweiner@rics.bwh.harvard.edu (H.L. Weiner).

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uterus a suspension of living cells from an adult mouse of a given strain to a fetal mouse of another strain, that when the inoculated mouse grew up, it was found to be partially or completely tolerant of skin grafts transplanted from any mouse belonging to the strain of the original donor. Thus, the hypothesis underlying this experiment is that "mammals and birds never develop, or develop to only a limited degree, the power to react immunologically against foreign homologous tissue cells to which they have been exposed sufficiently early in fetal life" [1].

This classic work, together with those from Owen and Hasek [7,9], defined our current understanding of the immunological basis of tolerance to self and foreign antigens. The definition of tolerance as a negative counterpart of immunity comes from the work of Burnet who first proposed self/non-self discrimination as a major principle driving the operation of the immune system and tolerance to self-components as a deletional event taking place at early periods of development [2]. According to Burnet's "Clonal Selection Theory", self-tolerance was based on the blindness of the mature immune system to body components. However, the description of the thymic selection of T lymphocytes, the subsets of T cells, including the regulatory T (Treg) cells, and their distinct actions, as well as the demonstration of autoreactive B and T cells in normal individuals contributed to change this scenario [10–13]. It became clear that natural tolerance to auto-components is a more complex phenomenon. Studies derived from this new approach clearly demonstrated that natural tolerance to self is an active process that depends on the activity of non-inflammatory/regulatory auto-reactive T lymphocytes present at a stable frequency in the normal repertoire [14–16]. In analogy to the natural non-inflammatory reactivity that the immune system mounts to self-components, the name "oral tolerance" has been given in the seventies to the immunological tolerance to antigens that access the body via the oral route [3].

2.2. Oral tolerance

In the beginning of the 20th century, Wells and Osbourne [17] demonstrated for the first time the phenomenon of immunological tolerance through oral fed antigens by showing that guinea pigs fed corn-containing diet, but not corn-free diets, were not anaphylactically sensitized to zen, a major protein of corn. Subsequently, several researchers showed that when mammals are exposed to foreign macromolecules by digestive route, delivered either by gastric intubation or ingested with maternal milk, they became immunologically tolerant to these proteins [18,19]. Moreover, David [20] showed that rats fed horse serum or pollen extracts were made tolerant to those antigens; Thomas and Parrot [21] tolerized rats by repeated feeding of bovine serum albumin; and Andre and co-workers [22] showed oral tolerance induction in mice fed sheep red blood cells. In the context of allergic disease, Vaz et al. [3] contributed significantly to the field by showing that intense and prolonged states of specific immunologic tolerance with profound specific IgE reduction could be induced in adult mice by a single exposure to OVA. Thus, these works demonstrated that, despite the fact that the vast majority of the macromolecules ingested as food are degraded within the gut, antigenically intact materials are absorbed in amounts sufficient to induce significant modifications in the immune responsiveness of the organism.

The demonstration of transferable cellular suppression associated with oral tolerance is a recurrent theme reported by many investigators [23]. Mowat et al. [24] reported that high doses of ovalbumin-induced tolerance were not abrogated by cyclophosphamide, which is believed to block active suppression, and that such tolerance affected antibody responses. Low doses of ovalbumin induced a state of tolerance that could be reversed by cyclophosphamide and primarily affected cell-mediated responses. Thus, this study already seemed to delineate active suppression vs. anergy depending on the dose of antigen (discussed below). Moreover, Hanson & Miller [25] reported two components of oral tolerance following oral administration of ovalbumin: tolerance was observed in both cyclophosphamide-treated and untreated animals, but tolerance from cyclophosphamide-treated animals could not be transferred.

Our early studies of oral tolerance in autoimmune models have found active suppression to be an important mechanism, and we have identified regulatory cells generated following oral tolerance that act via the secretion of antigen-non-specific regulatory cytokines triggered by the fed antigen [26]. Such cells were first characterized in the Lewis rat model of experimental autoimmune encephalomyelitis (EAE) orally tolerized to low doses of guinea pig MBP. The regulatory cells identified in that model were CD8+ T cells [27]; they transferred suppression in vivo and also suppressed in vitro; and acted via the secretion of TGF-β following antigen-specific triggering [28]. Further studies demonstrated that the epitopes of guinea pig MBP triggering CD8+ T regulatory cells following orally administered MBP were different from the encephalitogenic determinant [29]. Moreover, TGF-β-secreting regulatory cells could be found in Peyer's patches 24–48 h after one feeding of low doses of MBP, and these cells did not proliferate in response to 1 mg MBP even though they release TGF-β upon in vitro stimulation [30].

When similar studies were extended to a mouse system, it was found that CD4+ T cells were also responsible for active suppression, both in vivo and in vitro [31,32]. Thus, when a low dose of MBP was administered orally to SJL/J mice, Th1 but not Th2 immune responses were suppressed. In fact, Th2 cytokines (IL-4, IL-10) and TGF-β were significantly increased in mice fed with low doses of MBP. Furthermore, if animals were fed MBP and then immunized intraperitoneally with the same antigen, one enhanced the production of IL-4 and IL-10, as well as TGF-β. McGhee and colleagues also found that exposure of soluble antigens to the gut preferentially generated Th2-type responses as judged by increased IL-4 and IL-5 production [33]. Thus, in the gut, immune responses to soluble antigen are preferentially of a Th2 type and involve the generation of cells that secrete TGF-β. When cells from mice fed and immunized with MBP were further studied in vitro, it was found that both CD4+ and CD8+ T cells secreted TGF-β, whereas only CD4+ T cells secreted IL-4 and IL-10 [31]. However, it was clear in the SJL model that a population of TGF-β-secreting CD4+ T cells were also generated and amplified in the gut following the feeding and subsequent immunization with MBP.

CD4+ T cells were then cloned from the mesenteric lymph nodes of MBP-fed mice, and it was found that the majority of T-cell clones produced active TGF-β in addition to varying amounts of one or the other of the Th2-type cytokines (IL-4 and IL-10). However, it appeared that the TGF-β clones were different from classic Th2-type cells, as there was a general correlation between the secretion of IL-4 and IL-10 in an individual clone, whereas this was dissociated for TGF-β and IL-4/IL10. We named these cells as Th3 cells. Mucosal-derived CD4+ T cell clones were further characterized for their epitope specificity, MHC restriction and TCR usage. Sequence analysis of their cDNA revealed that they used Vα1 or Vα3, and Vβ4 or Vβ17, all of which were also used by encephalitogenic Th1 cells. Most interestingly, one of these mucosal Th3 clones used identical TCR Vα and Vβ chains as one of the encephalitogenic Th1 clones. The regulatory T cell clones generated in this study had striking similarities to the encephalitogenic CD4+ Th1-cell clones in terms of their specificity, TCR usage and MHC restriction. However, they could be distinguished from the encephalitogenic CD4+ Th1-cell clones by the fact that they produced suppressive cytokines (TGF-β, IL-4 and IL-10) following antigen-specific activation. The clones inhibited the proliferation and cytokine production of MBP-specific Th1 cells, and they suppressed the development of MBP-induced EAE and proteolipid protein-induced EAE, and this suppression was abrogated by in vivo injection of anti-TGF-β antibodies. This demonstrated that these clones were able to mediate bystander suppression in vivo mediated by TGF-β production [31].

The identification of the master transcription factor regulator Foxp3 in 2003 by Sakaguchi's group [34] (discussed below) shed light on how a CD4+ T cell, using identical variable TCR chains to an effector CD4+