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REVIEW

T cell tolerance to the skin: a central role for central tolerance

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Abstract T cell tolerance to self-antigens is believed to be achieved in a two-step process. The first step, called central tolerance, takes place in the thymus. The second step takes place outside the thymus in secondary lymphoid organs. One may ask why two mechanisms are needed to insure T cell tolerance. These two mechanisms share redundant functions and dysfunctions, leading to T cell-mediated autoimmune syndromes. By reviewing the literature on relevant animal models for T cell tolerance and our own recent findings, we are providing evidences that only central tolerance is acting for the skin.

Keywords Central tolerance · Peripheral tolerance · Skin · T cells

Introduction

An immune system is built so as to avoid mounting reactions against normal healthy cells, constituting the "self". Immune cells are educated not to recognise self-antigens. This education is based on the removal of cells that are too strongly recognising self-antigens from the active repertoire. Only cells with a low affinity for self-antigens are spared. These latter cells are expected to be of the strongest affinity for foreign antigens presented in the context of a self-major histocompatibility complex.

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For T cells, education is mediated in two anatomically separated steps. A first step, called central tolerance, occurs in the thymus. There, self-antigens expressed by medullary thymic epithelial cells (mTEC) are presented directly to maturating T cells. These antigens can also be presented at the surface of thymic-resident dendritic cells (DC) via a cross-presentation process [1]. A strong interaction between a TCR expressed by developing thymocytes and self-antigens leads to thymocyte cell death, development arrest and/or lineage diversion [2], eliminating these cells from the mature T cell repertoire. Obviously, this mechanism is efficient for epithelial antigens and tissue-restricted antigens (TRA) not expressed in mTEC have to be presented in the thymus to insure a more complete T cell tolerance to self. This can be performed in two ways. First, the very same mTEC have the unique property to express TRA, in a so-called promiscuous fashion, due to the expression of the specific transcription factor autoimmune regulator [3, 4]. Second, peripheral DC immigrating in the thymus can also cross-present TRA to maturating thymocytes [5]. Again, these two pathways of TRA presentation in the thymus lead to the deletion of the most avid T cells. A second step of T cell tolerance occurs on mature T cells in secondary lymphoid organs. It is called peripheral tolerance [6]. There, TRA are picked up by DC in tissues and cross-presented to naïve mature T cells in lymph nodes draining the corresponding tissue [7]. This can be done directly by the migrating DC or perhaps via a transfer of the antigen to a lymph-node-resident DC [8]. TRA recognition in peripheral lymph nodes conducts to T cell tolerance in the steady state. Here, tolerance is achieved either by cell deletion or by induction of an unresponsiveness state, called anergy [9], depending on the self-antigen doses [10]. At that stage, one may ask why two mechanisms are needed to insure T cell tolerance. Central and peripheral tolerance may appear

redundant, rather than being complementary. The absence of total complementarity between the two mechanisms is evidenced by the fact that T cell tolerance is not complete, sparing a repertoire of self-reactive cells harming the host when appropriately activated in the periphery. This is the case for patients suffering from insulin-dependent diabetes mellitus [11], rheumatoid arthritis [12] or multiple sclerosis [13, 14]. In these autoimmune pathologies, self-reactive T cells have been shown to attack their target organs, mediating loss of tissue integrity and function, thereby conducting to syndrome. In addition, these two tolerance pathways share redundant dysfunctions. They harbor the same leakiness in early life [15, 16].

In this study, we will focus on T cell tolerance to skin antigens. As for most if not all organs, T cell tolerance to skin is only partial because T cell-mediated autoimmune manifestations are clinically seen. However, it is not known at what level(s) the tolerance is failing. We have reviewed the current knowledge in T cell tolerance to skin antigens and attempted drawing conclusions for this organ.

Tolerance to keratinocyte antigens

The skin is composed of keratinocytes, target for self-reactive T cells in the autoimmune bullous pemphigoid [17]. In healthy individuals, tolerance mechanism(s) must act on T cells to avoid keratinocyte damages. Recently, transgenic mouse models based on the expression of a model antigen specifically in keratinocytes have shed some light on the pathway used to insure tolerance to keratinocyte antigens.

Three different groups have created a mouse expressing ovalbumin specifically in keratinocytes. This has been done by using regulatory elements from the keratin 14 (K14) [18, 19] or keratin 5 (K5) promoter [20]. Remarkably, these investigators observed unanimously that peripheral encounter of the self-antigen induced cytotoxic effector T cells. To our knowledge, this constitutes the first example of an efficient T cell priming upon recognition of a self-antigen in the periphery under steady-state conditions and, when numbers of specific T cells are kept low, close to physiological conditions. For self-antigens expressed elsewhere, T cell priming does not occur. Deletion is observed for antigens expressed in hematopoietic cells [21], prostate [22] and pancreas [23], while anergy or hypo-responsiveness is observed for intestine [24], liver [25] and brain [26] antigens. For eye antigens, a null event may follow peripheral T cell recognition of the self-antigen [27]. In the transgenic models investigated, T cells primed by keratinocyte antigens are pathogenic, causing damages to skin resembling graft vs host disease [18, 28] and/or toxic epidermal necrolysis [20]. Mechanistic studies have been performed in this model. Antigen-presenting cells involved in peripheral self-antigen presentation were dependent on the promoter used. It was found that radio-resistant Langerhans cells (LC) in the case of the K14 promoter [29] and radio-sensitive bone-marrow-derived DC in the case of the K5 promoter [20] were involved. In both cases, cross-presentation of the self-antigens picked up from keratinocytes were probed in skin-draining lymph nodes. This observation argues against the dogma that tissue dendritic cells migrating to draining lymph nodes in the steady state mediate T cell tolerance [30], at least for skin antigens. In the case of keratinocyte antigens, T cell priming in the periphery has to be counterbalanced to avoid the so-called horror autoxicus described early by Ehrlich referring to autoimmune reactions [31]. A regulatory pathway suppressing the function of the cytotoxic, skin-damaging, effector T cells induced has been observed [32]. This is mediated by a conditioning of LC into a tolerising state by responding T cells. However, this is acting too late after induction of cytotoxic T cells to avoid damage. In fact, central tolerance able to counteract the peripheral priming for a normal T cell repertoire acts for keratinocyte antigens [19, 20]. Taken together, these different models highlight the dominant role of central tolerance for keratinocyte antigens.

Tolerance to melanocyte antigens

The skin is also composed of melanocytes, which are lining the basal layer of keratinocytes. Melanocytes are also target for self-reactive T cells in an autoimmune pathology called vitiligo [33]. Again, the occurrence of such autoimmune reactions implies that melanocyte-specific self-reactive T cells are kept silent in healthy individuals. In an approach similar to the one described above for keratinocyte antigens, we have created a model to study T cell tolerance to tyrosinase, a melanocyte antigen. This was achieved by the generation of different mouse lines (Tyr-OVA mice) expressing a model antigen, ovalbumin, specifically in melanocytes [34]. In these transgenic animals, the model self-antigen was constantly presented to T cells in peripheral lymph nodes. Notably, this presentation neither involved radio-sensitive bone-marrow-derived cells nor radio-resistant Langerhans cells. Indeed, self-antigen presentation also occurred in lymph nodes distant from the skin, such as mesenteric lymph nodes, known to be devoid of immigrating skin Langerhans cells [35]. The cells involved in self-antigen presentation express tyrosinase, other melanocytic enzymes, such as Trp-1, Trp-2 and gp100, but are not pigmented. They are therefore likely to be lymph node-resident melanocyte not fully differentiated to produce pigment such as those in the skin. Our

observation is consistent with the fact that melanocytes resist irradiation (p. 517 of [36]). The presence of melanocyte in lymph nodes has already been pointed out in the case of nevi observed in this organ. These nevi, observed in lymph nodes draining a primary melanoma, are called nodal nevi [37]. They form compact aggregates of small cells, similar to cutaneous nevi, and therefore cytologically distinct from melanoma cells. Proximity of a primary tumor may induce proliferation of lymph-node-resident melanocytes. Less frequently, they are formed by an uncommon pigmented lesion, called blue nevus, developing in lymph nodes in the absence of cutaneous melanoma [38]. In this case, they may originate from the proliferation of melanocytes arrested during their differentiation in lymph nodes [39]. In mice, we observed melanocytes in skin-draining (inguinal and axillary) and mesenteric lymph nodes, from 1 to 11 per node section (Fig. 1). They are located in the sinus and the capsule, strongly suggesting that they are migrating via the lymphatic system. In some instances, we observed some of these cells lining the T cell area. Such a location is consistent with the site of nodal nevi occurrence. It is interesting to note that Slingluff et al. [40] has investigated in detail the mechanisms involved in T cell tolerance to endogenous tyrosinase. Notably, they also reported that tyrosinase presentation in skin-draining lymph nodes involved radio-resistant cells. We observed T cell priming upon peripheral antigen presentation, while they did not. This discrepancy may be due to affinity differences with a priming event for high-affinity T cells. We have used TCR-transgenic OT-I $CD8^+$ T cells, known to be of high affinity for their cognate OVA peptide [41]. Slingluff et al. have used newly generated tyrosinase-specific TCR transgenic $CD8^+$ T cells, of which affinity has not been reported yet. The priming we observed was efficient, as it induced fully differentiated cytotoxic effector $CD8^+$ T cells that persisted in lymph nodes. These cells did not induce autoimmune damage to skin melanocytes, as mice did not develop skin de-pigmentation characteristic for vitiligo. This is likely due to the absence of skin inflammation in our model. Skin inflammation has been shown by others to be required to initiate melanocyte destruction in animals containing a pool of melanocyte-specific cytotoxic T cells, such as after an active vaccination protocol against melanocyte antigens [42]. However, the presence of cytotoxic T cells in lymphoid organs represents a serious autoimmune threat that needs to be tightly controlled.

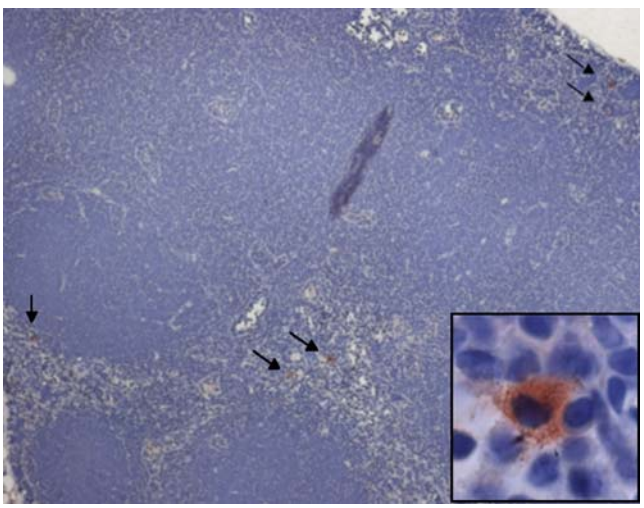

Fig. 1 Lymph-node-resident melanocytes. A skin-draining lymph node was immunostained with the polyclonal anti-tyrosinase pep7 antiserum [50]. Tyrosinase-expressing melanocytes are marked with an *arrow*. The *insert* is showing a high-power microscopy image of a stained cell

For tyrosinase used in our study, thymic deletion of specific T cells may represent a safe alternative to avoid T cell priming in the periphery. Indeed, we observed that 60% of the OVA-specific T cells are deleted in the thymus of double transgenic OT-I x Tyr-OVA mice. Thymic cell deletion was complemented by TCR tuning on an additional 20% of the cells, and only 20% of OVA-specific cells were able to escape central tolerance. This escape from central tolerance is likely due to the exaggerated number of specific T cells in thymi from double transgenic mice. Indeed, OVA-specific cells could neither be observed in the periphery from naïve nor after repeated vaccinations in single transgenic Tyr-OVA mice. This indicates that central tolerance was complete for an endogenous T cell repertoire. In the periphery, the escaping cells encountered the self-antigen in lymph nodes, became cytotoxic $CD8^+$ T cells and persisted similarly to adoptively transferred naïve T cells from mice not expressing the self-antigen (OT-I mice).

How is central tolerance to tyrosinase achieved? As discussed in the "Introduction", central tolerance can be achieved in three distinct ways: direct presentation by mTEC or cross-presentation by DC either resident in the thymus or immigrating from the periphery. Our present observations do not allow us to fully conclude. However, Slingluff et al. [40] performed thymus exchange between wild type (WT) and albino mice, deficient in the tyrosinase gene, to investigate the contribution of this organ in the establishment of tolerance to tyrosinase. They did not observe any influence of the thymus on the reactivity to tyrosinase for peripheral T cells. Thymectomised albino mice reconstituted with a WT thymus showed the same degree of peripheral reactivity than fully albino mice. In addition, grafting of thymi from albino mice to thymectomised WT mice did not allow the recovery of a high-affinity T cell reactivity in the periphery. It is unlikely that alternate cervical thymi [43, 44] could, on their own, replace cervical thymus function in this kind of experiment.

To our knowledge, such complementation of thoracic thymi by cervical thymi has never been reported. Experiments by Slingluff et al. indicate that tyrosinase expression in the thymus, despite having been probed in mTEC (our own observation and in [45]), unexpectedly does not serve any role in thymocyte education. Hence, central tolerance is likely to be executed by peripheral DC cross-presenting the self-antigen to thymocytes (Fig. 2). The central tolerance we have observed in our Tyr-OVA mice does not depend on the sub-cellular localisation of the self-antigen in melanocytes. It is as efficient for a secreted antigen than for an antigen expressed at the cell membrane and even for an antigen sequestrated into the cytosol. So is the priming observed in the periphery. Therefore, central tolerance may also be effective for a wide set of melanocyte antigens.

Concluding remarks

Achievement of T cell tolerance cannot be considered as optimal, as an important human population is suffering from diverse T cell-mediated autoimmune pathologies. As an example, vitiligo affects up to 2% of a homogeneous population [46]. Recent progresses made in animal models created to investigate T cell tolerance are telling us that T cell tolerance is a complex multi-step process, dependent on the organ considered. In this study, we provide compelling evidence that the skin is a special organ in terms of T cell tolerance. Indeed, presentation of self-antigens from skin keratinocytes or melanocytes resulted in the periphery in an efficient T cell priming. For these self-antigens, central tolerance is a dominant mechanism.

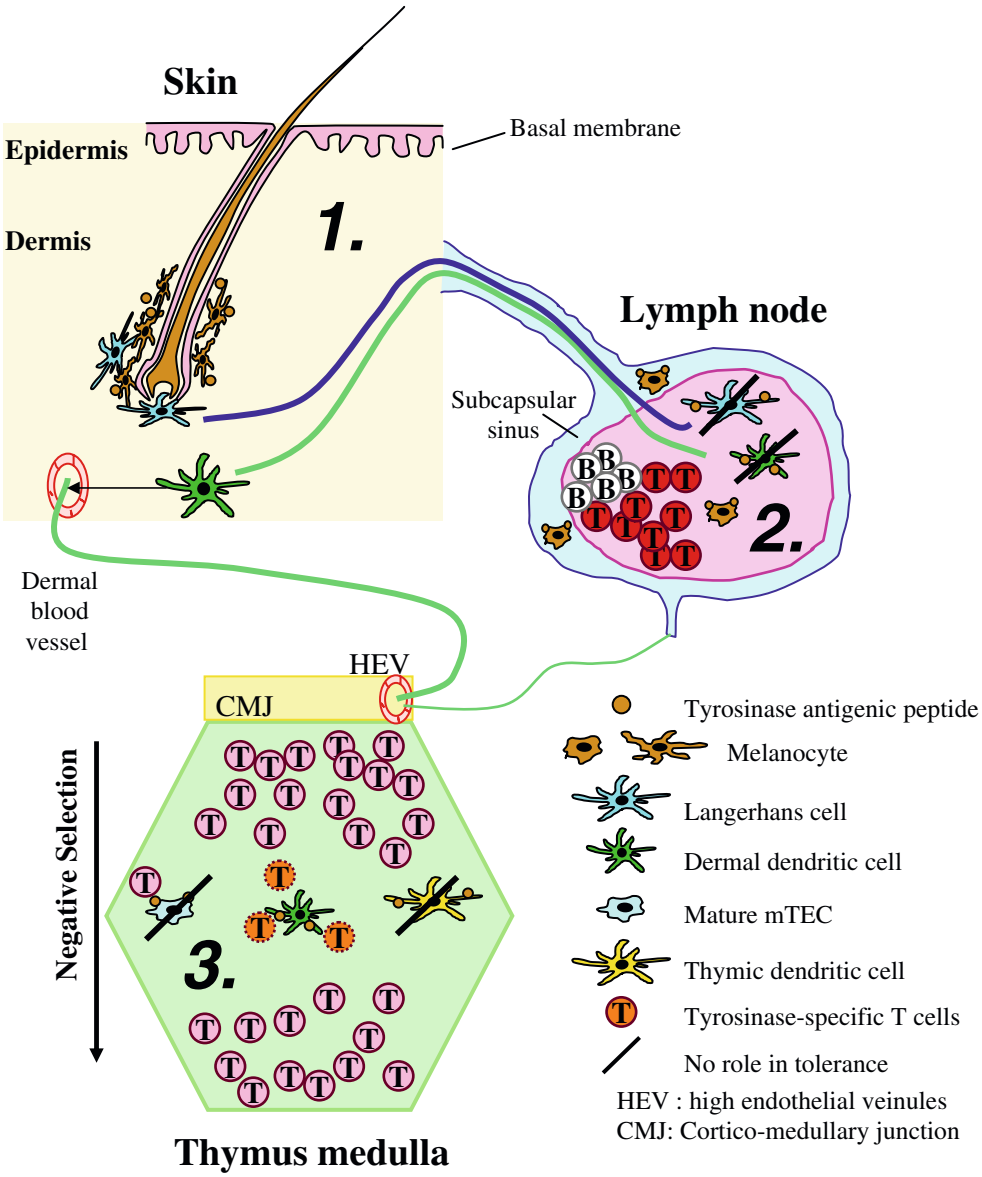

Fig. 2 Postulated central pathway mediating T cell tolerance to tyrosinase. *1* Melanocytes are localised at the base of hair follicles. LC and dermal DC can pick up antigens from melanocytes and migrate to the draining lymph nodes. *2* Within the draining lymph nodes, immigrating loaded LC, dermal DC and lymph-node-resident melanocytes have no obvious role in the induction of peripheral tolerance to tyrosinase. *3* Peripheral DC loaded with tyrosinase antigens can migrate in the thymus and eliminate maturing T cells presenting a too-high-affinity TCR for tyrosinase. In the thymus, mTEC expressing tyrosinase and resident DC have no obvious role in the central tolerance to tyrosinase

What are these new findings telling us? It is telling us that vitiligo is likely to originate from a defect in central tolerance that must be combined to an environmental factor leading to skin inflammation, owing to the requirement of the latter factor to start the damaging process. It will be of interest to evaluate whether central tolerance defect is similar among all individuals, rendering environmental factor(s) the only variable, or whether central tolerance efficacy is variable between individuals. The observations presented herein are also raising doubts regarding the existence of peripheral tolerance to skin antigens. TRA are presented in the periphery in the steady state to survey for mature T cells newly emigrating from the thymus with potential self-reactivity due to central tolerance escape. In this steady-state condition, there is no infection, consequently no danger signal and no strong co-stimulatory activity of DC [47]. Hence, steady-state presentation should induce T cell tolerance. However, any event leading to DC activation in the periphery during TRA presentation may change a tolerising event into a priming event, leading to the generation of effector self-reactive T cells able to damage tissues. Autoimmune reactions induced by bystander DC activation have been demonstrated in several animal models [48], and we know that we are too much exposed to pathogens to believe that such bystander events are rare. Taken together, this leads to the idea that mechanisms acting for peripheral tolerance are not mediated without any risk for the host. In contrast, central tolerance by presentation of self-antigens to thymocytes appears less risky. There bystander activation of DC, either thymic resident or immigrating from the periphery, should even lead to an increased central tolerance based on the requirement of co-stimulatory signals for this process [49]. Based on all these considerations, the skin, exposed to many infections and therefore containing bystander-activated DC, may have evolved in a way to suppress peripheral tolerance pathways compared to other organs less exposed to infection.

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