

Lymphocyte unresponsiveness

with

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What does anergy mean?

Vigo Heissmeyer: The term anergy describes a phenomenon observed in self-reactive lymphocytes that enter a state of functional unresponsiveness. The importance of this process in T cells becomes clear from the significant proportion of antigen receptors found on peripheral T cells that display reactivity against self-antigens. These cells arise since the mechanisms of central tolerance are not fail-safe: The process of positive selection initially selects T cells with receptors that are able to signal in the absence of foreign antigens and the following process of negative selection only deletes overtly self-reactive T cells that recognize the self-antigens that are presented in the thymus. The immune system therefore depends on additional mechanisms in the periphery that prevent or dampen the unwanted responses against self. T cell anergy is a cell intrinsic mechanism that involves partial, suboptimal stimulation; for example the engagement of the antigen receptor in the absence of costimulation. When the T cell receives such anergizing stimulation it becomes over time unresponsive to restimulation through the T cell receptor as measured by the lack of IL-2 production and proliferation. One molecular feature of anergic T

cells is that upon restimulation they display defects in proximal T cell receptor signal transduction. These defects can be bypassed experimentally through stimulation with pharmacological agents that activate downstream effectors of T cell activation and restore IL-2 production and T cell proliferation.

The molecules that render T cells unresponsive *in vivo* and *in vitro* have been intensively sought-after. Strategies of differential expression analysis have been employed, since it is widely accepted that unresponsiveness is achieved by the transcriptional changes that happen during the induction phase of anergy. Recently, we and others have identified several E3 ubiquitin ligases as important factors in T cell unresponsiveness, since the upregulation or membrane raft localization and functional importance in anergic T cells could be demonstrated (1-3). Therefore we have proposed that molecules that are involved in proximal TCR signal transduction are targeted in anergic T cells by the action of membrane localized E3 ubiquitin ligases so that the stimulation through the T cell receptor will fall below the threshold of signal strength that is required for productive activation (4).

What is the role of NFAT in T cell anergy?

Vigo Heissmeyer: NFAT appears to play a very interesting dual role in T cells. When T cells become stimulated through the TCR, the stimulation strength is translated into the sustained elevations of intracellular calcium which leads to NFAT activation after dephosphory-

lation by the Ca²⁺-dependent phosphatase calcineurin. In the process of productive activation NFAT forms a cooperative complex with AP-1 and this complex is critical for the induction of effector cytokine genes like *IL2* (5). However knockout studies demonstrated

that NFAT not only induces activation and proliferation, but also exerts negative regulation since T cells from NFAT1 knockouts show hyper-proliferation after T cell receptor stimulation (6). These findings indicate that besides its function in T cell activation, NFAT also instructs negative feed-

back. In fact constitutive NFAT activity in T cells inhibits cytokine gene expression and many anergy associated target genes are NFAT dependently regulated (7). It is therefore possible that NFAT or specific NFAT complexes are master regulators of the anergy program in T cells.

How is anergy linked to other mechanisms of peripheral tolerance?

Vigo Heissmeyer: The two major constituents of peripheral tolerance are T cell anergy and T cell regulation/suppression and it is a challenge to find out whether there is a link between both. Regulatory T cells have been found to be critical in the prevention of many autoimmune diseases. The development and function of these T cells depend on a lineage determining transcription factor Foxp3 (8,9). Several possible links need to be addressed: Are the mechanisms that are responsible for unresponsiveness in anergic and in suppressed T cells similar? Recent work has proven that besides natural regulatory T cells that are generated in the thymus, adaptive regulatory T cells can also be induced in the periphery (10). It is therefore interesting to ask: Can anergic T cells become regulatory T cells or can they

induce the development of effector T cells into the T reg lineage? This conversion would generate a 'memory' for self-antigen by generating long-lived regulatory T cells. The fact that regulatory T cell properties can be induced in the periphery by stimulation of CD4 T cells with TGF-beta that triggers FoxP3 expression (11) gives us a starting point to address such questions. Moreover it is known that regulatory T cells are specific for self-antigens and that it is required to stimulate regulatory T cells to bring about their regulatory/suppressive activity. It therefore seems likely that self-antigen stimulation of the T cell receptor in regulatory T cells induces the transcription factor NFAT and that this factor, being already important in T cell activation and T cell anergy, serves another specific function in regulatory T cells.

Can your findings be used for any therapeutic benefit?

Vigo Heissmeyer: In principle the modulation of anergy bears enormous potential. Therapeutic induction of the anergy program could be used to prevent transplant rejection or to treat autoimmune disease without the need to broadly suppress the immune system. On the other hand to interfere with established anergy could be a tool to make the immune system reactive against tumor cells that escaped immune surveillance. However at present we still need to gain more insight into the molecular

details of the anergy program and to find effective small molecule inhibitor that are able to silence very specifically a defined molecular function.

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