still able to proliferate. Thus, paracrine IL-2 produced "in trans" by the host T cells can act with CD28 signals delivered "in cis" to drive *in vivo* proliferation of transferred IL-2 KO CD4⁺ T cells after injection of mAb D665. Since IL-2 is known to mediate survival and activation of Treg cells, the abundant production of IL-2 *in vivo* together with stimulatory CD28 signals may explain the preferential expansion of Treg cells by CD28 superagonists.

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Q.4 B cell activation and affinity discrimination by receptor segregation?

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B cell interaction with antigen results in an affinity-dependent response. Unlike other receptors, the B cell receptor (BCR) can be triggered by a large number of different ligands (antigens) and discriminates between a wide range of binding affinities. The mechanisms by which the mechanical interaction with antigen is translated into a cellular signal in an affinitydependent manner is so far unknown. Here it is proposed that the experimentally observed segregation of the BCR from inhibitory proteins triggers B cell signalling and enables affinity discrimination. By building a mathematical model, whose parameters are all determined by experimental data, we show that B cell activation by inhibitior receptor segregation is in agreement with available in vitro data and explains the (previously counterintuitive) ranges of affinity discrimination that are observed in experiments (Batista and Neuberger, 1998&2000). Moreover, the model offers an explanation for why haptens are only eliciting a B cell response when bound to a larger carrier. The model also provides an insight as to how the reduced IgM surface expression observed on anergic B cells may cause B cell unresponsiveness.

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Q.5 Costimulation induced phosphorylation of L-plastin is important for surface expression of the T cell activation markers CD69 and CD25

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Cooperative signals from the antigen-specific TCR/CD3complex and costimulatory receptors like CD2 or CD28 are essential to express the functional repertoire of T cells. In the absence of costimulation, antigen recognition can lead to T cell anergy or apoptosis. Costimulatory signals may ,therefore, represent valuable targets for therapeutic immune modulation. Here we demonstrate that the actin-bundling protein L-plastin is phosphorylated upon costimulation of human peripheral blood T lymphocytes (PBT) via TCR/CD3 plus CD2 or CD28, respectively but not following triggering of each of these receptors alone. Mass spectrometry and site directed mutagenesis showed that Ser-5 represents the only phospho-acceptor site of L-plastin in human PBT. Interestingly, expression of a non-phosphorylatable S5A-L-plastin mutant exhibited a dominant negative effect on the activation of PBT. Thus, cells expressing the 5A-L-plastin mutant showed a significantly lower expression of the T cell activation markers CD25 and CD69 on the cell surface than cells expressing wildtype-Lplastin. This effect is due to a failure in the transport of CD25 and CD69 to the cell surface. In conclusion, enabling the transport of activation receptors to the cell surface through Lplastin phosphorylation represents a so far unknown mechanism by which costimulation controls the activation of T cells.

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Q.6 Cytohesin-1 and Cytohesin-3 have antagonistic roles in T-cell activation

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The ability of T-cells to respond to T cell receptor engagement is a complex mechanism tightly controlled by a variety of intracellular signaling pathways which are still not fully understood. Here we show that the cytoplamic protein Cytohesin-1 functions as a signaling factor in T-cell activation. By the use of the siRNA approach we demonstrate that Cytohesin-1 signals to the IL-2 promoter via AP-1 and NFkappaB. On the other hand NFAT mobilization was not affected by Cytohesin-1 silencing. These results suggests that Cytohesin-1 may be required for T-cell activation. We have previously shown that the homologous protein Cytohesin-3 is strongly upregulated in anergic T-cells. Therefore we investigated a potential involvement of Cytohesin-3 in T-cell activation signaling. Interestingly, we found that Cytohesin-3 abrogates IL-2 promoter activation by inhibiting the AP-1 and NFkappaB signaling branch. However, NFAT dependent signaling events were also repressed by Cytohesin-3. In line with these data, we demonstrate that Cytohesin-3 is upregulated in an ex-vivo antigen-dependent tolerance model. We purpose that Cytohesin-3 inhibits T-cell activation at an early stage and may thus be an important factor for anergy/tolerance induction.

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Q.7 Downmodulation of CD86 in antigen presenting cells by the mouse Cytomegalovirus immunomodulatory gene modB7-2 is dependent on proteasome function

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Mouse Cytomegalovirus (MCMV) not only modulates the innate immune response but also targets the cells that play a