Abstract# 3498

LYMPHOPROLIFERATIVE AND AUTOIMMUNE DISORDER IN MICE WITH NULL FUNCTION OF GDP-DISSOCIATION INHIBITOR OF RHO-GTPASES. Guosheng Lin*,[1] Luk V. Parijs*,[2] Wei Hu*,[1] Xiaoyu Li*,[1] Jun L. Zhou*,[3] Janet Buhlman*,[1] Satoshi Takamatsu*,[1] Timothy Chevassut*,[1] Keith Elkon*,[3] Bing Lim.[1] [1]*Medicine, Harvard Medical School, Boston, MA, USA;* [2]*Cell Biology, California Institute of Technology, Pasadena, CA, USA;* [3]*Medicine, Cornell University Medical College, New York, NY, USA.*

The Rho-sub-family of small GTPases(Rho, Rac, CDC42) has been shown to play crucial roles in multiple fundamental cellular functions. These GTPases work as on/off switches by cycling between inactive GDP-bound and active GTP-bound forms.One of the key proteins regulating this switch is the Rho GDP-Dissociation Inhibitors (RhoGDIs). Three RhoGDIs have thus far been found. GDI-D4 is a GDI that is expressed at high levels preferentially in hematopoietic cells. To understand better the in vivo function of GDIs, we have generated murine animals with knock-out of GDI-D4 gene. About 20% of -/- animals develop massive generalized lymphadenopathy and splenomegaly with the onset of clinical disease ranging from 2 months to as old as a year. No disease have been observed in over 200 +/+ and 50+/- mice followed over 2 years.The disease progressed rapidly and animals usually die within 2-3 months after developing adenopathy. Histopathology revealed lymphoid hyperproliferation with increased plasma cells in lymph nodes and spleen. Tissue sections of kidneys showed glomerulo- nephropathy with immune- complex deposits in the glomeruli. Animal sera showed polyclonal gammopathy with high titers of anti-nuclear and anti-double stranded DNA antibodies. In advanced disease multiple organs are infiltrated with lymphocytes and myeloid cells. FACS analysis revealed increased expression of Fas (CD95) in B cells and all Thy1+ cells are CD44+/CD62-, a hall-mark of activated memory T cells. About 30% of young mice were found to have abnormal CD44hi T cells before development of clinical adenopathy. Preliminary studies revealed that T cells of diseased animals are resistant to activation and to Fas-induced apoptosis. The pathology appears to be a defect in T cell development and function, resulting in T cells that fail to kill B cells including auto-reactive clones, leading to an expansion of B cells, plasma cells and development of autoimmune diseases. How the null-function of GDI-D4 leads to the defect in T cell function and why not all -/- animals develop the disease remains to be investigated. Our results therefore point to an important role of RhoGTPase pathways in the development and function of T cells. These results also demonstrate a crucial in vivo role of the GDIs in modulating the activities of RhoGTPases.

Abstract# 3499

REGULATION OF IFN-γ GENE EXPRESSION BY NFAT1. Alexander Kiani*,[1] Francisco J. Garcia-Cozar*,[2] Gerhard Ehninger,[1] Anjana Rao*.[3] [1]*Medizinische Klinik und Poliklinik I, Universitaetsklinikum Carl Gustav Carus, Dresden, Germany;* [2]*Department of Biochemistry and Molecular Biology, Faculty of Sciences, Cadiz University, Cadiz, Spain;* [3]*The Center For Blood Research and the Department of Pathology, Harvard Medical School, Boston, MA, USA.*

Transcription factors of the nuclear factor of activated T cells (NFAT) family are thought to regulate the T cell receptor (TCR)-inducible, cyclosporin A-sensitive expression of a variety of genes in T cells, including those encoding IL-2, IL-4, TNF-α and FasL. However, the role of NFAT proteins in regulating transcription of the IFN-γ gene remains unclear. Here we show that NFAT1 is a major regulator of TCR-induced IFN-γ production in T cells. Compared to cells expressing NFAT1, both $CD4^+$ and $CD8^+$ T cells freshly isolated and purified from NFAT1-deficient mice display a profound defect in the TCR-induced expression of IFN-γ mRNA and protein; in contrast, an alternative IL-12- and IL-18-dependent pathway of IFN-γ production is unaffected. TCR-induced IFN-γ production is reduced in naive as well as in differentiated T cells lacking NFAT1, and cannot be fully rescued even when differentiation is done under Th1-polarizing conditions. Reduced IFN-γ expression in NFAT1-deficient cells is not secondary to overproduction of the Th2 cytokine IL-4, as the study was performed with mice bred on the $IL\text{-}4^{-/-}$ background; and it is not secondary to overexpression of the Th2-specific transcription factors c-Maf and GATA-3 which are known suppressors of IFN-γ production. Additionally, IFN-γ production in a murine T cell clone was found to be markedly susceptible to the recently described selective peptide inhibitor of NFAT, VIVIT. In summary, our results suggest that NFAT1 regulates IFN-γ expression at the level of acute gene transcription in naive and differentiated T cells. Considering the central role of T cell-derived IFN-γ in areas such as tumor immunology, infection and Graft-versus-Host-Disease, the emerging prospective of new drugs designed to specifically target NFAT proteins may have impact on several areas in the field of hematology.

Abstract# 3500

Tob, A NOVEL Tob/BTG1 FAMILY MEMBER THAT ASSOCIATES WITH THE CCR4 TRANSCRIPTIONAL REGULATORY COMPLEX IS SELECTIVELY EXPRESSED IN ANERGIC T CELLS AND INHIBITS IL-2 TRANSCRIPTION. Dimitrios Tzachanis*,[1] Naoto Hirano*,[1] Gordon J. Freeman*,[1] Lee M. Nadler,[1] Vassiliki A. Boussiotis.[1] [1]*Adult Oncology, Dana-Farber Cancer Institute, Boston, MA, USA.*

Induction of T cell anergy is an active signaling process that requires calcium release and protein synthesis. The known biochemical characteristics of anergy involve differential activation of proteins that are constitutively expressed.Since induction of anergy is an active process, de novo mRNA synthesis or gene transcription will also occur.To identify genes selectively induced in anergic cells we employed the subtractive suppression hybridization (SSH) approach. SSH is based on exponential amplification of sequences, which differ in abundance in two compared populations, whereas the amplification of sequences of similar abundance is suppressed. T cell clones were anergized by anti-CD3, controls were rescued from anergy by CD3+CD28 mAb, mRNA was isolated and SSH was performed between cDNA from control and anergic cells. A total of 200 individual clones were analyzed. 30 clones were selectively upregulated in anergic cells. Sequence analysis showed matches with known genes for 21 clones. Here we describe Tob (transducer of Erb-B) a gene selectively expressed in anergic cells. Tob is a member of the novel Tob/BTG1 family that consists of BTG1, PC3/TS1/BTG2, Tob and ANA/BTG3. These proteins have a homologous N-terminal domain, which interacts with a component of the CCR4 transcription factor that binds cdk4 and inhibits G1 phase cell cycle progression. To examine the role of Tob on IL-2 gene transcription, Jurkat T cells were transfected with full length Tob cDNA and the IL-2 promoter/enhancer DNA(2kb) linked to the luciferase(luc) gene. Tob inhibited CD3+CD28-mediated IL-2 transcription by 98%. The Jun family of transcription factors bind to the IL-2 promoter and activate IL-2 transcription. Analysis of nuclear extracts showed that Tob did not affect induction of c-Jun, c-Fos or phosphorylation of c-Jun.To determine whether inhibition of IL-2 transcription was mediated by defective transactivation of known IL-2 promoter elements even though these transcription factors were present, AP-1-luc, NF-AT-luc and NF-kB-luc were used. Surprisingly, Tob did not inhibit transcription of any of these reporters. These results strongly suggest that the negative regulatory effect of Tob on IL-2 transcription is not mediated by defective expression or transactivation of known IL-2 transcription factors but is mediated through an active suppressive effect on the IL-2 promoter. Identification of the cis and trans-acting elements via which Tob negatively regulates IL-2 gene transcription will define a target for molecular intervention towards tolerance induction.

Abstract# 3501

CTLA4 HAS AN OBLIGATORY ROLE FOR THE INDUCTION OF TOLERANCE OF NAIVE T CELLS IN VIVO. Vassiliki A. Boussiotis,[1] Rebecca J. Greenwald*,[2] Alla Berezovskaya*,[1] Abul K. Abbas*,[3] Arlene H. Sharpe*.[2] [1]*Adult Oncology, Dana-Farber Cancer Institute, Boston, MA;* [2]*Brigham and Women's Hospital, Harvard Medical School, Boston, MA;* [3]*Department of Immunology, UCSF, San Francisco, CA.*

Elucidating the physiologic mechanisms that govern induction and maintenance of tolerance in vivo will provide insights into the pathophysiology of autoimmunity and facilitate the design of novel therapies for induction of T cell tolerance.The fatal lymphoproliferation of $CTLA4^{-/-}$ mice supports a critical role for CTLA4 in downregulating T cell activation. Therefore, we examined the effect of CTLA4 on the decision of naive T cells between activation and tolerance. To generate a population which exclusively expresses a specific T cell receptor (TCR) that allows antigen-specific stimulation in vivo, we generated $DO11^+RAG2^{-/-}CTLA4^{-/-}$ mice. DO11 mice express a MHC class II-restricted transgenenic (Tg) TCR specific for $OVA_{323\text{-}339}$ peptide. Use of the $DO11^+RAG2^{-/-}CTLA4^{-/-}$ ($CTLA4^{-/-}Tg$) and the $DO11^+RAG2^{-/-}$ (WT-Tg) allows the comparison of naive cells that differ only in their ability to express CTLA4. T cells from WT-Tg and $CTLA4^{-/-}Tg$ mice were adoptively transferred into syngeneic WT recipients, which were either left untreated (naive) or immunized with $OVA_{323\text{-}339}$ subcutaneously (primed) or intravenously (tolerized). TCR-Tg cells were harvested from draining nodes and stimulated with $OVA_{323\text{-}339}$ to determine response on rechallenge. Naive WT-Tg and $CTLA4^{-/-}Tg$ cells showed comparable proliferation and IL-2 production. WT-Tg and $CTLA4^{-/-}Tg$ cells from the primed treatment group had significant proliferation and IL-2 and IFNg production, whereas $CTLA4^{-/-}Tg$ cells also produced IL-4. Strikingly, in the tolerized treatment group although WT-Tg cells proliferated poorly and did not produce IL-2 and IFNg, $CTLA4^{-/-}Tg$ cells showed significant proliferation and production of IL-2, INFg and IL-4. Cells of all treatment groups entered the G1 phase of the cell cycle. Naive and primed WT-Tg and $CTLA4^{-/-}Tg$ cells downregulated $p27^{kip1}$, activated cdk2, phosphorylated Rb and progressed through the cycle. WT-Tg cells from the tolerized treatment group were arrested at the G1 phase since they had high $p27^{kip1}$, and neither activated cdk2 nor phosphorylated Rb. In contrast, $CTLA4^{-/-}Tg$ cells from the tolerized treatment group downregulated $p27^{kip1}$, which allowed cdk2 activation, Rb phosphorylation and entry to the S phase. These results demonstrate a key role for CTLA4 in tolerance induction in vivo and have important implications for the development of immune based therapies involving manipulation of CTLA4 mediated signaling.

Abstract# 3502

ENGAGEMENT OF THE PD-1 IMMUNOINHIBITORY RECEPTOR BY A NOVEL B7-FAMILY MEMBER LEADS TO NEGATIVE REGULATION OF LYMPHOCYTE ACTIVATION. Gordon J. Freeman*,[1] Andrew J. Long*,[2] Yoshiko Iwai*,[4] Yvette Latchman*,[3] Karen Bourque*,[2] Julia A. Brown*,[1] Vassiliki A. Boussiotis,[1] David M. Dorfman*,[3] Tatyana Chernova*,[1] Hiroyuki Nishimura*,[4] Lori Fitz*,[2] Nelly Malenkovich*,[1] Taku Okazaki*,[4] Michael Byrne*,[2] Heidi Horton*,[2] Lynette Fouser*,[2] Laura Carter*,[2] Arlene H. Sharpe*,[3] Beatriz Carreno*,[2] Mary Collins*,[2] Clive R. Wood*,[2] Tasuku Honjo*.[4] [1]*Department of Adult Oncology, Dana-Farber Cancer Institute, Boston, MA, USA;* [2]*Genetics Institute, Inc., USA;* [3]*Brigham and Women's Hospital, Boston, MA, USA;* [4]*Kyoto University, Kyoto, Japan.*

PD-1 is a cell surface receptor structurally related to CD28 and CTLA4 and is expressed on activated lymphoid and myeloid cells. PD-1 contains an ITIM motif and has a role in the negative regulation of immune responses. Mice deficient in PD-1 have multiple autoimmune features and appear to have a breakdown in peripheral tolerance. We have identified a novel member of the B7 gene family, PD-L1, as the ligand of PD-1. PD-L1 does not bind to CD28, CTLA4, or ICOS. PD-L1-Ig fusion protein or PD-L1 transfected cells inhibit TCR-mediated activation of both human and murine T-cells but not PD-1 deficient murine T cells. Engagement of PD-1 by PD-L1 dramatically inhibits TCR stimulated proliferation and cytokine production by T cells but does not increase cell death. IL-2, IFN-γ, and IL-4