Class 15, BBS821: Control of pathogenic self-reactive T cells by co-inhibitory molecules, J. Kang
Oct 29, 2015

CTLA-4 regulates pathogenicity of antigen-specific autoreactive T cells by cell-intrinsic and -extrinsic mechanisms

Wataru Ise…… and Kenneth M. Murphy, Nat Immunol 2011

CTLA-4 Control over Foxp3$^+$ Regulatory T Cell Function

Kajsa Wing,[1*] Yasushi Onishi,[1,2] Paz Prieto-Martin,[1] Tomoyuki Yamaguchi,[1] Makoto Miyara,[1] Zoltan Fehervari,[1] Takashi Nomura,[1] Shimon Sakaguchi[1,3,4]†

Science, 2008

Immune dysregulation in human subjects with heterozygous germline mutations in *CTLA4*

Hye Sun Kuehn,[1*] Weiming Ouyang,[2*] Bernice Lo,[3,4*] Elissa K. Deenick,[5,6] Julie E. Niemela,[1] Danielle T. Avery,[5] Jean-Nicolas Schickel,[7] Dat Q. Tran,[8] Jennifer Stoddard,[1] Yu Zhang,[4,9] David M. Frucht,[2] Bogdan Dumitriu,[10] Phillip Scheinberg,[10] Les R. Folio,[11] Cathleen A. Frein,[12] Susan Price,[3,4] Christopher Koh,[13] Theo Heller,[13] Christine M. Seroogy,[14] Anna Huttenlocher,[14,15] V. Koneti Rao,[3,4] Helen C. Su,[4,9] David Kleiner,[16] Luigi D. Notarangelo,[17] Yajesh Rampertaap,[18] Kenneth N. Olivier,[18] Joshua McElwee,[19] Jason Hughes,[19] Stefania Pittaluga,[16] Joao B. Oliveira,[20] Eric Meffre,[7] Thomas A. Fleisher,[1]† Steven M. Holland,[4,18] Michael J. Lenardo,[3,4]† Stuart G. Tangye,[5,6] Gulbu Uzel[18]†

Science, 2014

Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial

Robert, C. et al. 2014 Lancet

Tolerance

- Tolerance - the absence of response to a specific antigen
- Also defined as “not doing harm to oneself”
  - Can have a response with no deleterious outcome
- Tolerance is antigen-specific: T and B cells

Autoimmunity: Loss of tolerance

- Normal individuals have self-reactive peripheral B and T cells
  - peripheral self-antigens that are not present during lymphocyte development and therefore do not initiate clonal deletion of specific lymphocytes

- Activation of self-reactive lymphocytes can lead to pathogenesis -> Autoimmunity

Tolerance occurs during lymphocyte development

- 'Central' tolerance
  - Clonal deletion (negative selection) to eliminate highly self-reactive lymphocytes
    - T cells: thymus
    - B cells: bone marrow
  - Clonal anergy - does not eliminate self-reactive lymphocytes but they become non-responsive
    - B cells: bone marrow

Central Tolerance is not enough?

- Antigens present in the thymus induce clonal selection
  - Self-proteins induce negative selection
    - self-proteins expressed in thymus
    - self-proteins in the circulation that get to the thymus
- TCR repertoire with high affinity for self is deleted

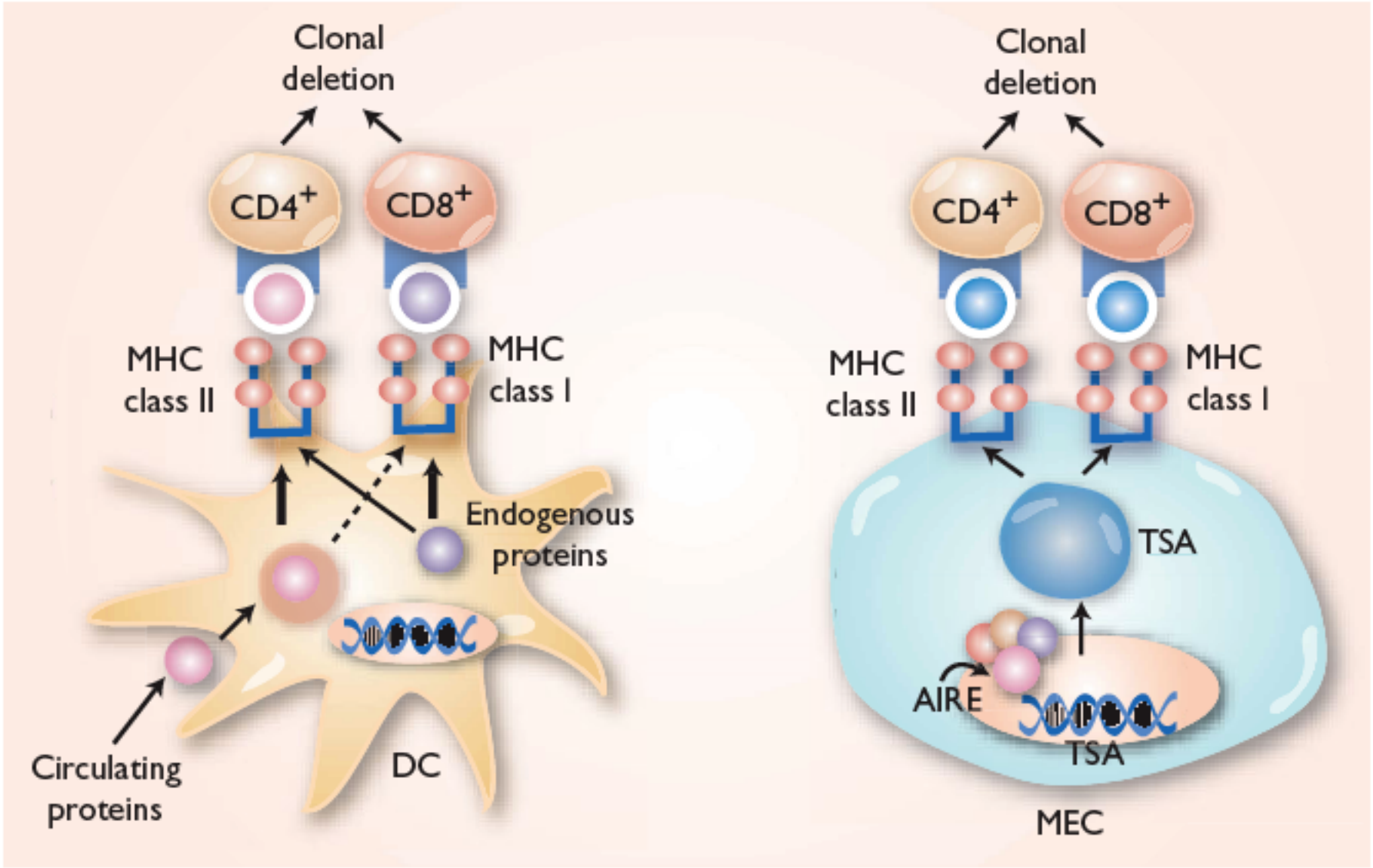
But…

- Naive peripheral T cells
  - Encounter self-proteins not seen in the thymus
  - Express TCRs with intrinsic affinity for self-MHC
  - Require survival signals via self-MHC plus peptide (low density or low affinity)

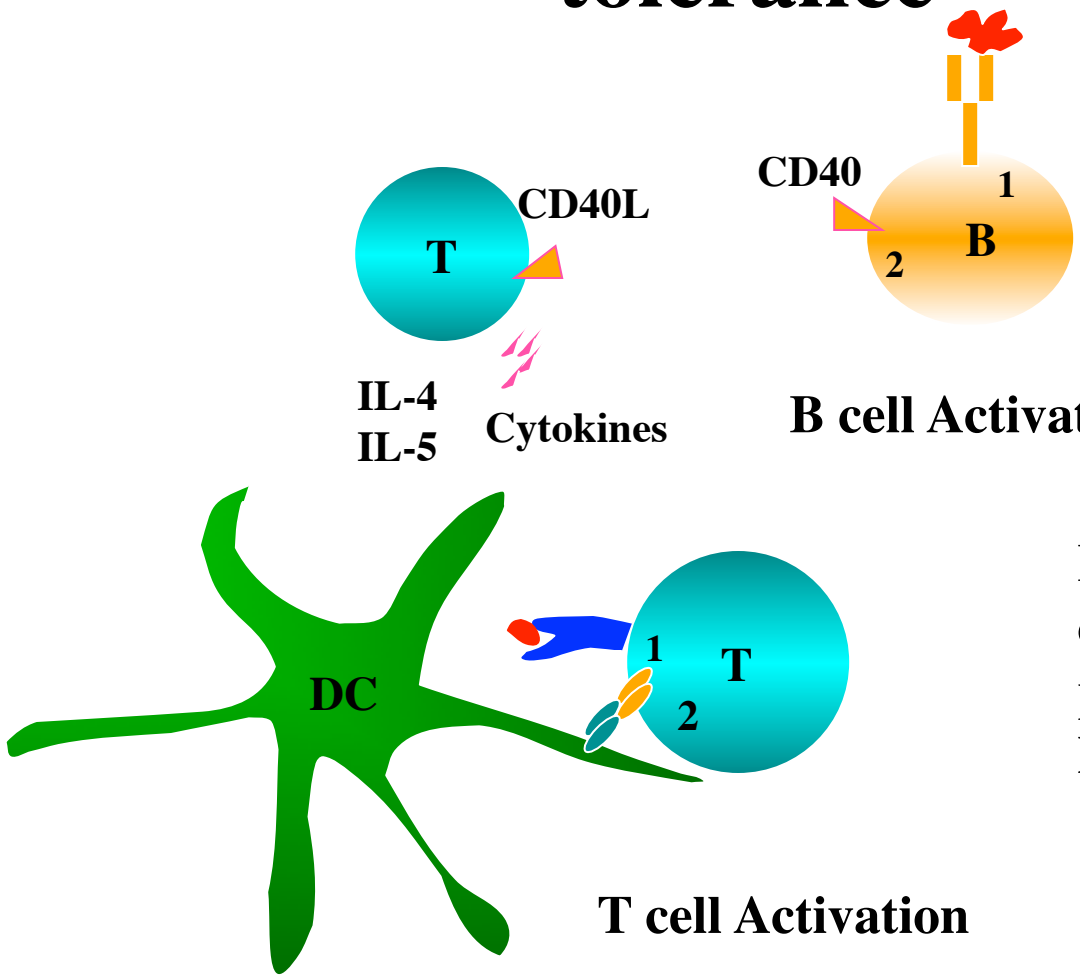
Common mechanisms of peripheral lymphocyte tolerance

- Anergy/hyporesponsiveness
- Ignorance
  - insufficient positive signals
  - dominant inhibitory signals
- Clonal deletion
- Immune Deviation
- Inhibition by specialized regulatory T cells

Presentation of self-proteins by BM-derived APCs vs MECs

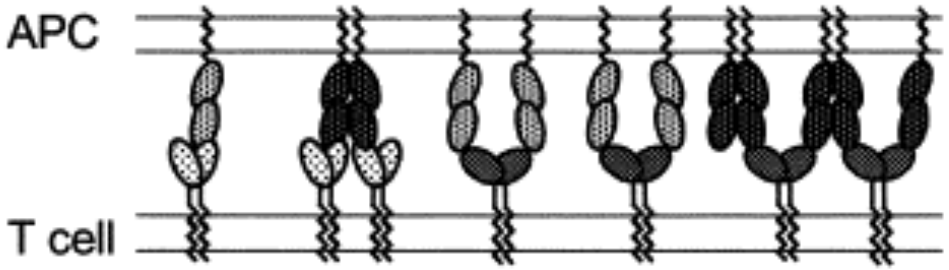


The requirement for two signals for lymphocyte activation is critical for tolerance



Increases in Signal 1 or Signal 2 can potentially lead to loss of tolerance

Signal 2: COSTIMULATION
CTLA-4 binds to B7 with higher affinities than CD28



	B7-2: CD28	B7-1: CD28	LICOS: ICOS	B7-2: CTLA-4	B7-1: CTLA-4
K_d (μM)	20	4	4	2.6 22.4	0.2 1.4
k_{on} ($M^{-1}s^{-1}$ X10^{-6})	≥1.4	≥0.66	N.D.	1.96	≥2.15
k_{off} (s^{-1})	≥28	≥1.6	N.D.	5.1	≥0.43

Biacore measurements, Collins et al. 2002 Immunity

Why two signals for activation?

- Lymphocytes specific for peripheral self-antigens due to lack of clonal deletion encounter tissue-specific antigens in the peripheral tissues
- Signal 2 is altered by changes in the microenvironment eg. inflammation
- Increased affinity and/or altered BCR specificity by somatic hypermutation during ongoing immune responses

CTLA-4 Control over Foxp3$^+$ Regulatory T Cell Function

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Science, 2008

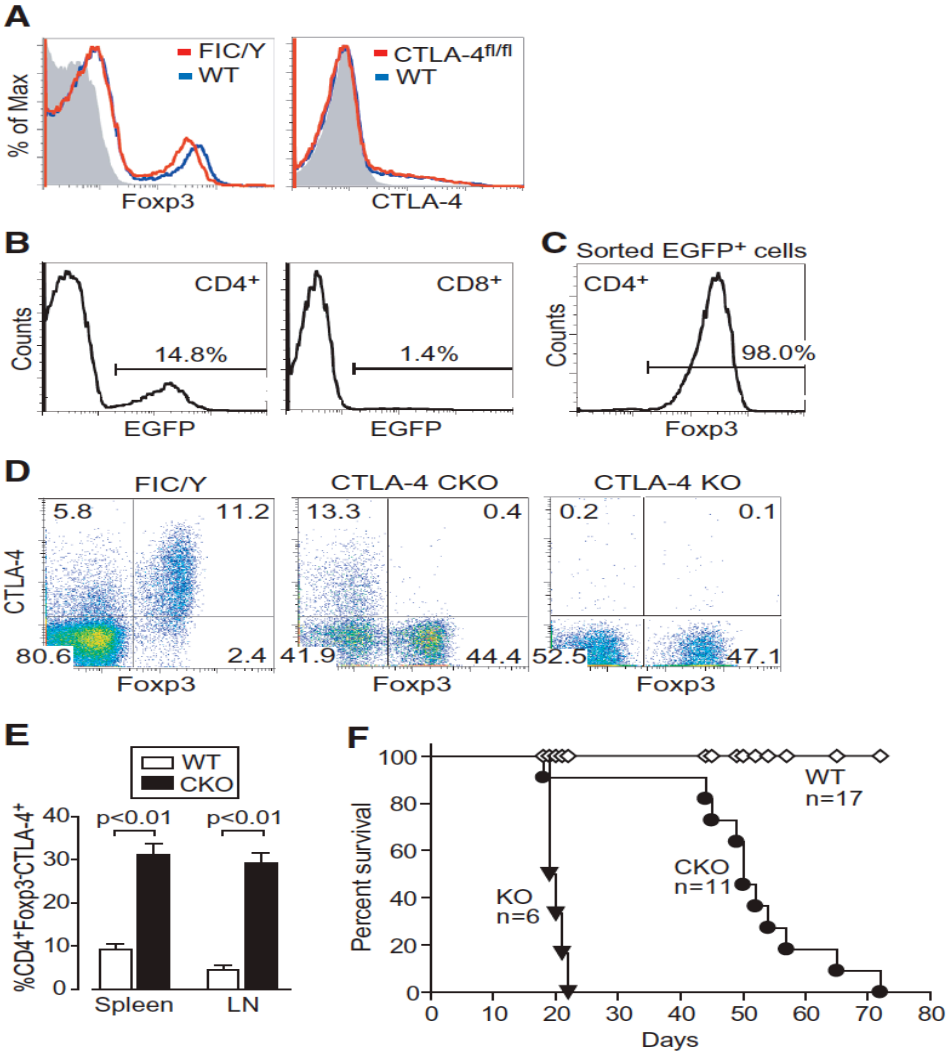


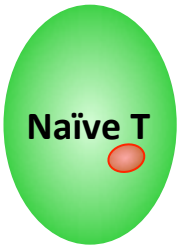
Fig. 1. Specific deletion of CTLA-4 expression in Foxp3$^+$ T cells results in fatal disease. **(A)** Flow cytometric analysis of intracellular Foxp3 (left) and CTLA-4 (right) in freshly isolated LN CD4$^+$ T cells from male FIC, CTLA-4$^{fl/fl}$, or BALB/c WT mice. **(B)** EGFP expression in CD4$^+$ or CD8$^+$ T cells derived from male FIC-CAG mice. **(C)** Sorted CD4$^+$EGFP$^+$ cells in FIC-CAG mice were stained for Foxp3. **(D)** CTLA-4 and Foxp3 expression in LN CD4$^+$ T cells from BALB/c WT, CKO, or KO mice. **(E)** Frequency of CTLA-4–expressing CD4$^+$Foxp3$^-$ T cells in CKO and normal littermates (n = 5). **(F)** Survival of KO and CKO mice as compared with normal littermates. Data represent three or more independent experiments. Vertical bars indicate SEM.

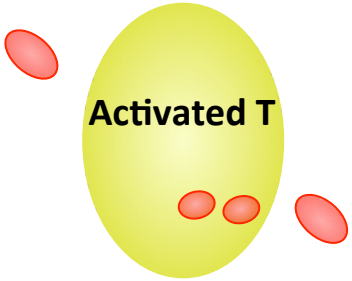
FIC foxp3-ires-cre
CAG Cre reporter

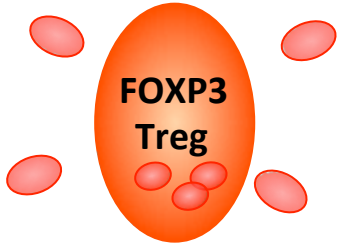
CTLA-4 function

Jain et al. PNAS 2010

Friedline et al. JEM 2009







Thymic selection?

TCR repertoire shaping?
Contraction of expansion

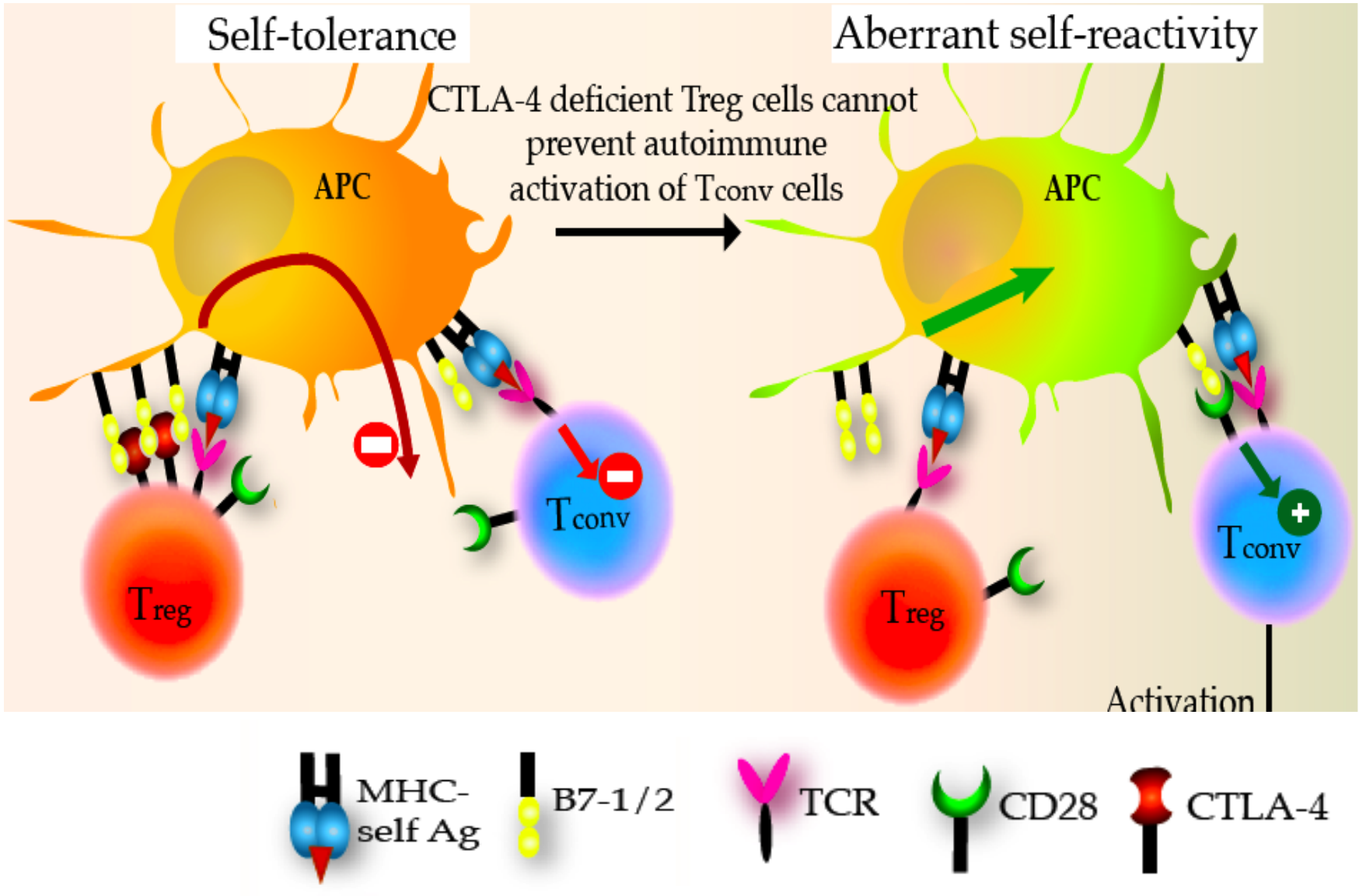
Maintenance of naïve T cell tolerance and homeostasis

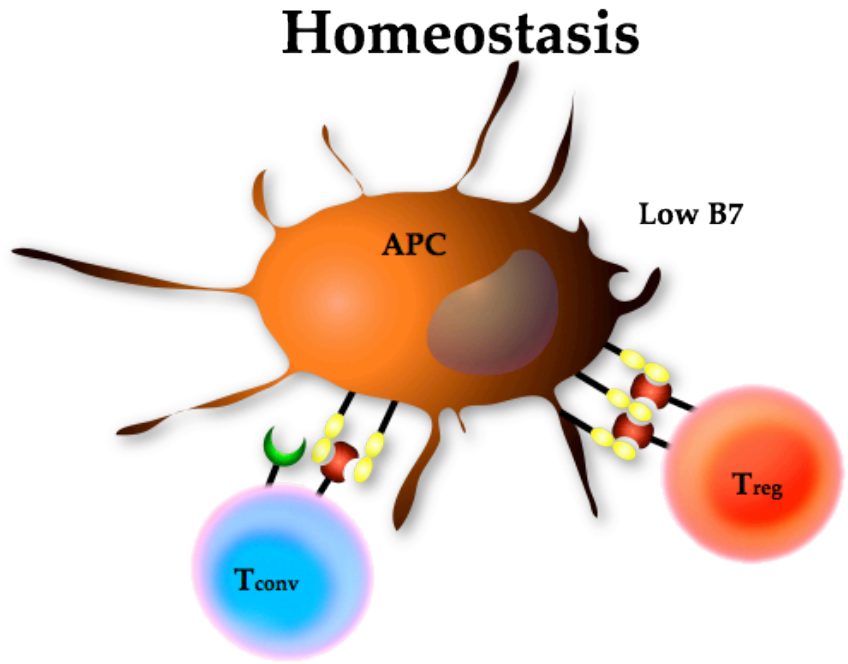
Fail-safe rerouting of self-reactive T cells?

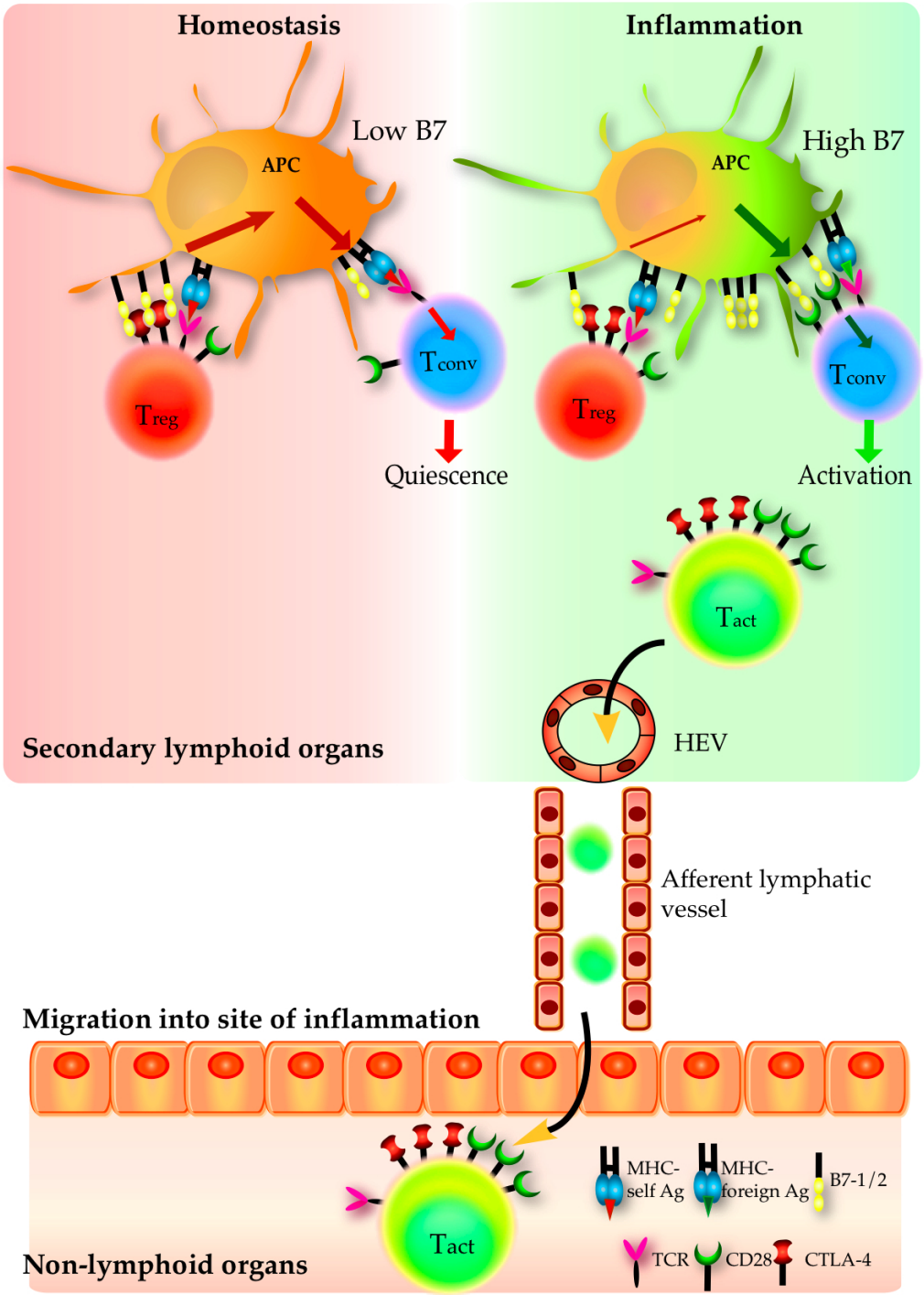
Surface CTLA-4

i.c. CTLA-4

Treg cells maintain naïve T cell quiescence via CTLA-4




Homeostasis
APC
Low B7
Treg
Tconv
Quiescence
Inflammation
APC
High B7
Treg
Tconv
Activation


Homeostasis
Inflammation
Low B7
High B7
APC
APC
Tconv
Treg
Treg
Tconv
Quiescence
Activation
Tact
HEV
Secondary lymphoid organs
Afferent lymphatic vessel
Migration into site of inflammation
Tact
MHC-self Ag
MHC-foreign Ag
B7-1/2
TCR
CD28
CTLA-4
Non-lymphoid organs

Immune dysregulation in human subjects with heterozygous germline mutations in *CTLA4*

Hye Sun Kuehn,[1*] Weiming Ouyang,[2*] Bernice Lo,[3,4*] Elissa K. Deenick,[5,6] Julie E. Niemela,[1] Danielle T. Avery,[5] Jean-Nicolas Schickel,[7] Dat Q. Tran,[8] Jennifer Stoddard,[1] Yu Zhang,[4,9] David M. Frucht,[2] Bogdan Dumitriu,[10] Phillip Scheinberg,[10] Les R. Folio,[11] Cathleen A. Frein,[12] Susan Price,[3,4] Christopher Koh,[13] Theo Heller,[13] Christine M. Seroogy,[14] Anna Huttenlocher,[14,15] V. Koneti Rao,[3,4] Helen C. Su,[4,9] David Kleiner,[16] Luigi D. Notarangelo,[17] Yajesh Rampertaap,[18] Kenneth N. Olivier,[18] Joshua McElwee,[19] Jason Hughes,[19] Stefania Pittaluga,[16] Joao B. Oliveira,[20] Eric Meffre,[7] Thomas A. Fleisher,[1]† Steven M. Holland,[4,18] Michael J. Lenardo,[3,4]† Stuart G. Tangye,[5,6] Gulbu Uzel[18]†

Cytotoxic T lymphocyte antigen–4 (CTLA-4) is an inhibitory receptor found on immune cells. The consequences of mutations in *CTLA4* in humans are unknown. We identified germline heterozygous mutations in *CTLA4* in subjects with severe immune dysregulation from four unrelated families. Whereas *Ctla4* heterozygous mice have no obvious phenotype, human *CTLA4* haploinsufficiency caused dysregulation of FoxP3$^+$ regulatory T (T_{reg}) cells, hyperactivation of effector T cells, and lymphocytic infiltration of target organs. Patients also exhibited progressive loss of circulating B cells, associated with an increase of predominantly autoreactive CD21^{lo} B cells and accumulation of B cells in nonlymphoid organs. Inherited human *CTLA4* haploinsufficiency demonstrates a critical quantitative role for CTLA-4 in governing T and B lymphocyte homeostasis.

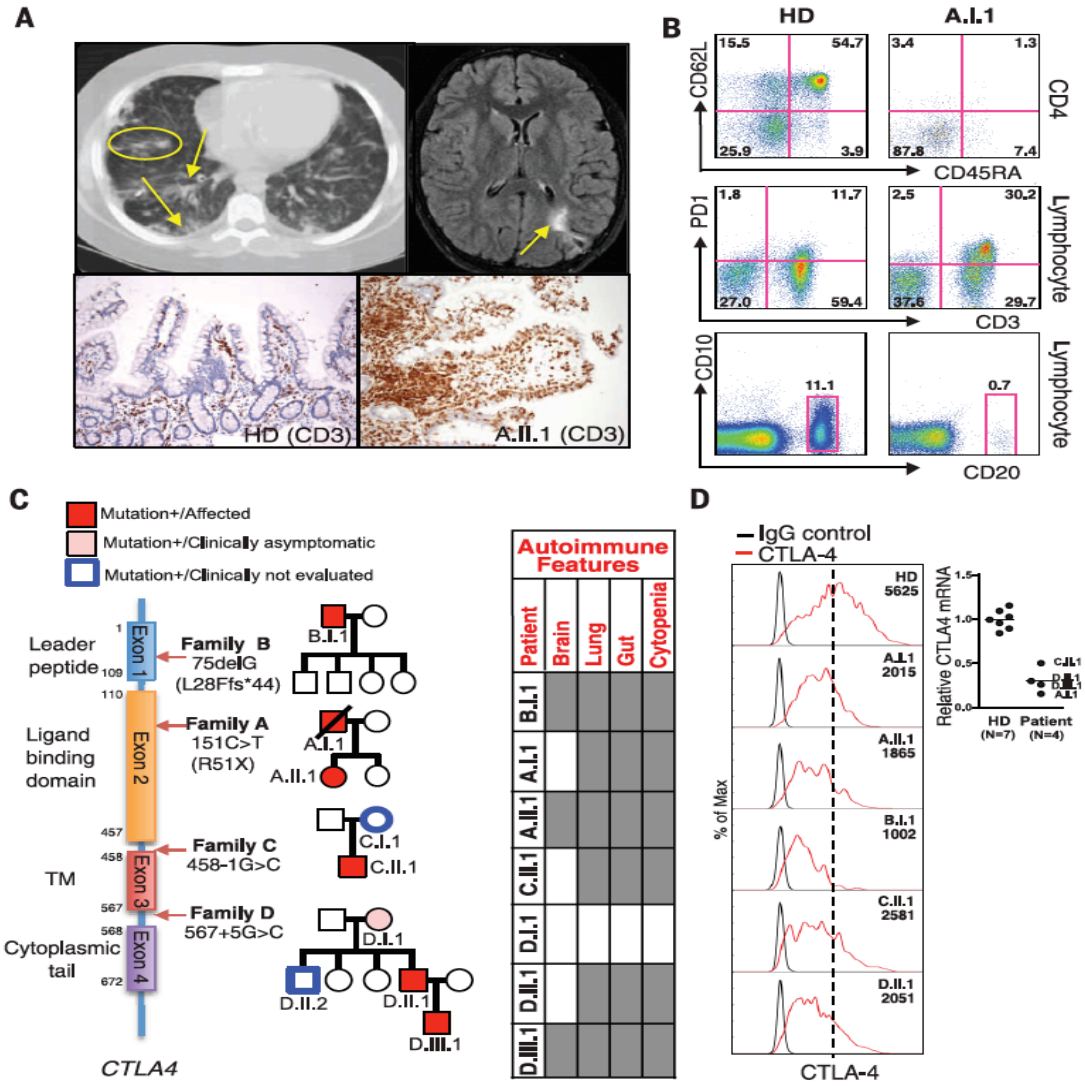


Fig. 1. Clinical phenotype and pedigree of the patients. (**A**) Top: Computed tomography images of lung and brain from patient A.II.1. Bottom: Histological section (magnification 20×) from a duodenal biopsy from a healthy donor (HD) and patient A.II.1 stained for CD3 (brown cells), showing an increased number of transepithelial T cells within the villi. (**B**) Flow cytometric analyses of $CD4^+$ cells or total lymphocytes stained for the indicated surface markers from a healthy donor and patient A.I.1. Data showing decreased $CD45RA^+CD62L^+$ naïve $CD4^+$ T cells are representative of three patients (A.I.1, A.II.1, and B.I.1). Programmed cell death–1 (PD1) expression data shown are representative of five patients (A.I.1, A.II.1, B.I.1, C.II.1, and D.II.1) and three healthy donors. Data showing decreased circulating B cells are representative of two patients (A.I.1 and A.II.1). (**C**) Mutations in patient alleles displayed on a schematic of the four exons of *CTLA4*, pedigrees, and phenotype summary highlighting organs (gray) with inflammatory infiltrates and autoimmune cytopenias for affected family members. TM, transmembrane domain. (**D**) Protein and mRNA expression of CTLA-4 in T_{reg} cells. Left: Levels of CTLA-4 expression in T_{reg} cells ($CD4^+CD25^+FoxP3^+$) were assessed by intracellular staining. The numbers in the upper right corner depict mean fluorescence intensity (MFI) of anti-CD152 (CTLA-4) staining. Dotted line indicates the peak of CTLA-4 expression in a healthy donor. Data shown are representative of three experiments. Right: Levels of *CTLA4* mRNA in T_{reg} cells ($CD4^+CD25^+CD127^{lo}$) sorted from seven different healthy donors and four patients were measured by real-time PCR using the probe for *CTLA4* transcript variant 1 (full length) and normalized to GAPDH. Data are means of replicates from six experiments. For relative gene expression, all data were normalized to the same HD. The horizontal lines indicate mean values from healthy donors or patients.

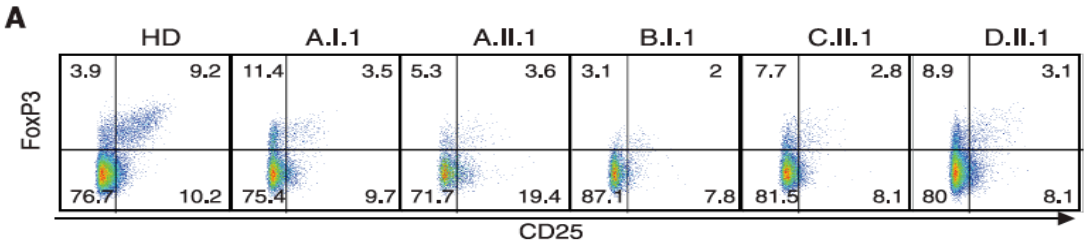
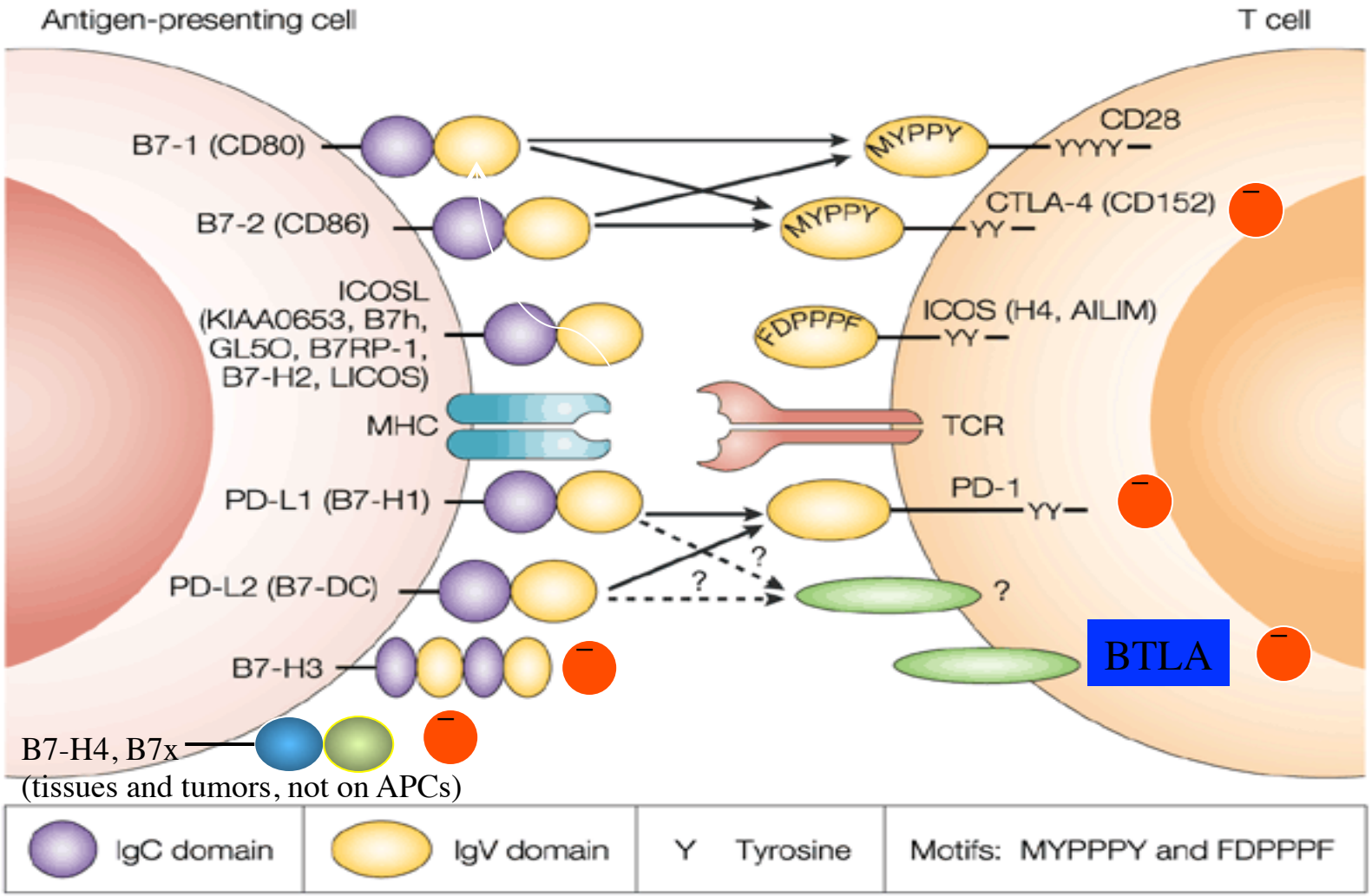
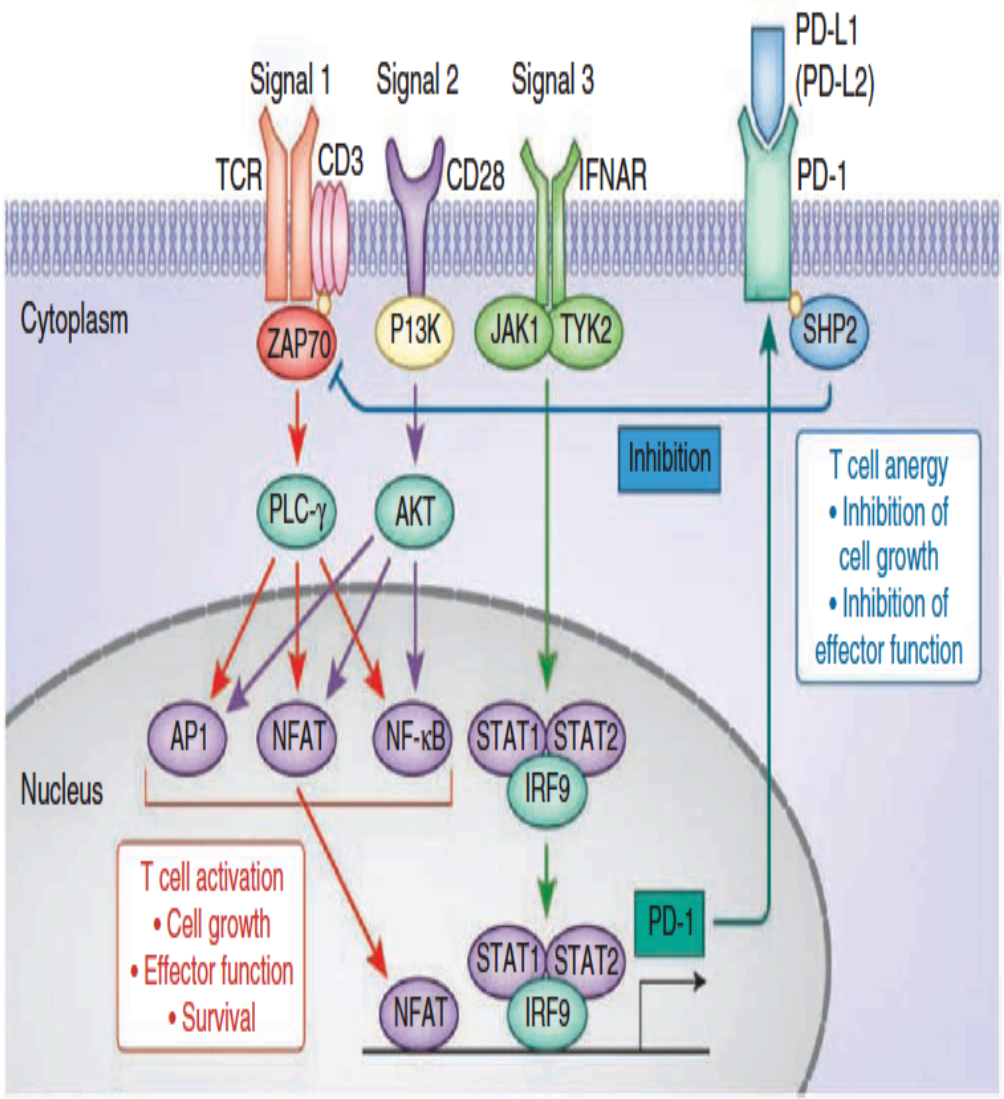


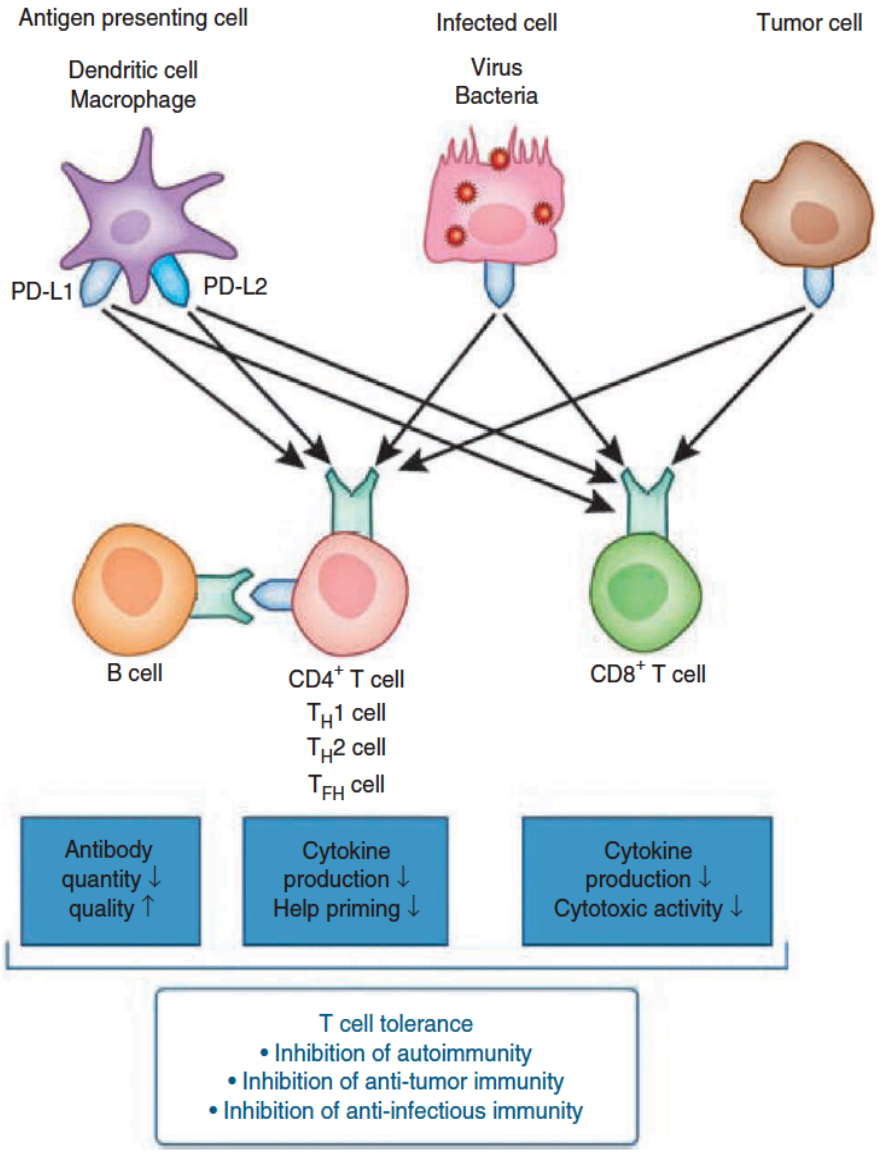
Fig. 2. Abnormal T_{reg} cell phenotype and function in patients. (**A**) Flow cytometric analysis of FoxP3 and CD25 in $CD4^+$ T cells from healthy donor (HD) and patients. (**B**) Mean fluorescence intensity of FoxP3 and CD25 in $CD4^+$ $FoxP3^+$ T cells from healthy donors and patients. Data are means ± SEM of replicates of indicated patient [A.I.1 (N = 7), A.II.1 (N = 1), B.I.1 (N = 2), C.II.1 (N = 2), D.II.1 (N = 4)] and 10 healthy donors. The N values represent number of



Modified from Freeman and Sharpe, 2003 **Nature Reviews | Immunology**

PD-1, activation-induced analog inhibitor of T cell function





Okazaki…..Honjo, NRI 2013

Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial

Robert, C. et al. 2014 Lancet

Summary

Background

The anti-programmed-death-receptor-1 (PD-1) antibody pembrolizumab has shown potent antitumour activity at different doses and schedules in patients with melanoma. We compared the efficacy and safety of pembrolizumab at doses of 2 mg/kg and 10 mg/kg every 3 weeks in patients with ipilimumab-refractory advanced melanoma.

Methods

In an open-label, international, multicentre expansion cohort of a phase 1 trial, patients (aged ≥18 years) with advanced melanoma whose disease had progressed after at least two ipilimumab doses were randomly assigned with a computer-generated allocation schedule (1:1 final ratio) to intravenous pembrolizumab at 2 mg/kg every 3 weeks or 10 mg/kg every 3 weeks until disease progression, intolerable toxicity, or consent withdrawal. Primary endpoint was overall response rate (ORR) assessed with the Response Evaluation Criteria In Solid Tumors (RECIST, version 1.1) by independent central review.

Findings

173 patients received pembrolizumab 2 mg/kg (n=89) or 10 mg/kg (n=84). Median follow-up duration was 8 months. **ORR was 26% at both doses—21 of 81 patients in the 2 mg/kg group and 20 of 76 in the 10 mg/kg group (difference 0%, 95% CI −14 to 13; p=0·96).** Treatment was well tolerated, with similar safety profiles in the 2 mg/kg and 10 mg/kg groups and no drug-related deaths. The most common drug-related adverse events of any grade in the 2 mg/kg and 10 mg/kg groups were fatigue (29 [33%] vs 31 [37%]), pruritus (23 [26%] vs 16 [19%]), and rash (16 [18%] vs 15 [18%]). Grade 3 fatigue, reported in five (3%) patients in the 2 mg/kg pembrolizumab group, was the only drug-related grade 3 to 4 adverse event reported in more than one patient.

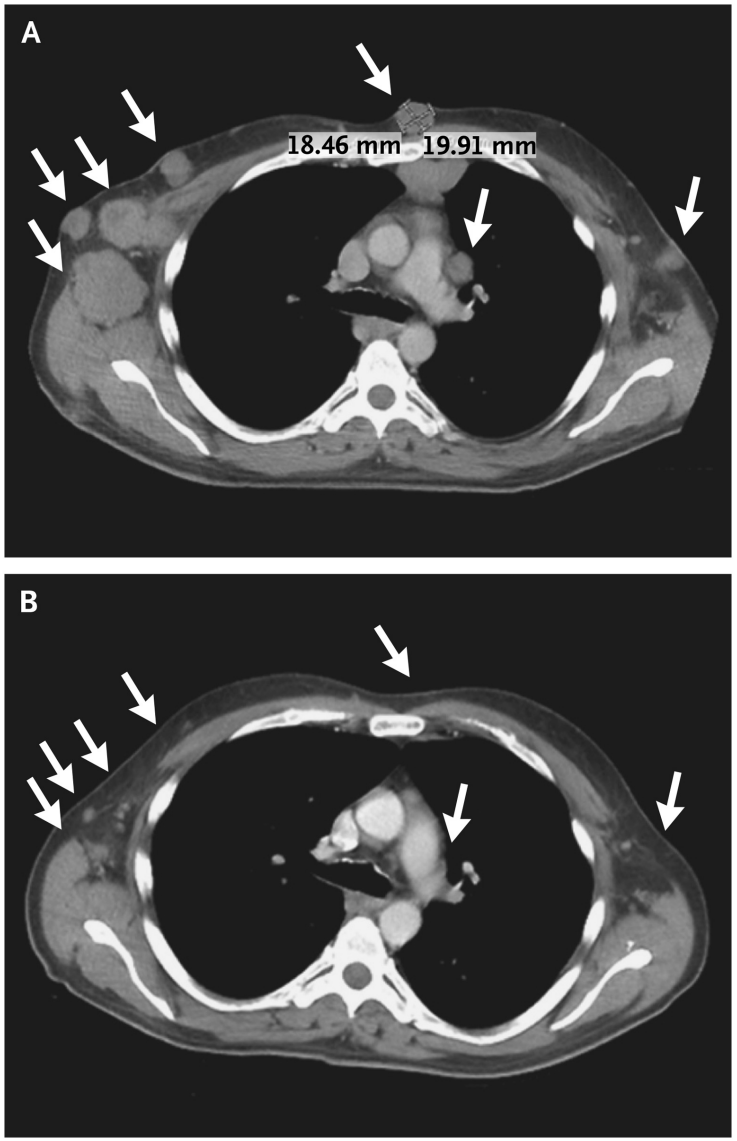
Interpretation

The results suggest that pembrolizumab at a dose of 2 mg/kg or 10 mg/kg every 3 weeks might be an effective treatment in patients for whom there are few effective treatment options.

ORIGINAL ARTICLE
Nivolumab (anti-PD-1) plus Ipilimumab in Advanced Melanoma

Wolchok et al.
N Engl J Med 2013; 369:122-133July 11, 2013

"53% of patients had an objective response, all with tumor reduction of 80% or more"



Computed Tomographic (CT) Scans of the Chest Showing Tumor Regression in a Patient Who Received the Concurrent Regimen of Nivolumab and Ipilimumab

Published September 14, 2015 // JEM vol. 212 no. 10 1603-1621

Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity

Alison M. Paterson,1,2,* Scott B. Lovitch,1,2,3,* Peter T. Sage,1,2 Vikram R. Juneja,1,2 Youjin Lee,2 Justin D. Trombley,1,2 Carolina V. Arancibia-Cárcamo,4 Raymond A. Sobel,5 Alexander Y. Rudensky,6 Vijay K. Kuchroo,2 Gordon J. Freeman,7 and Arlene H. Sharpe1,2