25. Shoop, R. D. *et al.* Synaptically driven calcium transients via nicotinic receptors on somatic spines. *J. Neurosci.* **21,** 771–781 (2001).
26. Cohen, P. S. *et al.* The critical role of p38 MAP kinase in T cell HIV-1 replication. *Mol. Med.* **3,** 339–346 (1997).
27. Andersson, U. *et al.* High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J. Exp. Med.* **192,** 565–570 (2000).

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Activation of human CD4$^+$ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype

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The immune system must distinguish not only between self and non-self, but also between innocuous and pathological foreign antigens to prevent unnecessary or self-destructive immune responses. Unresponsiveness to harmless antigens is established through central and peripheral processes[1]. Whereas clonal deletion and anergy are mechanisms of peripheral tolerance[2,3], active suppression by T-regulatory 1 (Tr1) cells has emerged as an essential factor in the control of autoreactive cells[4]. Tr1 cells are CD4$^+$ T lymphocytes that are defined by their production of interleukin 10 (IL-10)[5] and suppression of T-helper cells[6]; however, the physiological conditions underlying Tr1 differentiation are unknown. Here we show that co-engagement of CD3 and the complement regulator CD46 in the presence of IL-2 induces a Tr1-specific cytokine phenotype in human CD4$^+$ T cells. These CD3/CD46-stimulated IL-10-producing CD4$^+$ cells proliferate strongly, suppress activation of bystander T cells and acquire a memory phenotype. Our findings identify an endogenous receptor-mediated event that drives Tr1 differentiation and suggest that the complement system has a previously unappreciated role in T-cell-mediated immunity and tolerance.

Control of self-reactive T lymphocytes by regulatory T cells has been proposed to mediate anergy and thereby peripheral tolerance and prevention of autoimmunity[7]. Such cells suppress immune responses through either direct cell–cell interactions or the release of inhibitory cytokines such as IL-10 and transforming growth factor-β (TGF-β)[6,8–11]. The differentiation of CD4$^+$ lymphocytes into Tr1 cells is poorly defined, in part because of difficulties in inducing and culturing such cells. Only after the stimulation of human CD4$^+$ T cells with allogeneic monocytes or murine CD4$^+$ T lymphocytes with antigen were some Tr1 clones generated; however, these cells had weak proliferative capacities and required the presence of IL-10 (ref. 5). Tr1 cell differentiation has also been induced by incubating CD4$^+$ T cells with dexamethasone and vitamin D3 (ref. 12).

Defining the stimuli that drive differentiation of these cells would significantly enhance our understanding of their ontogeny and capabilities. Here we identify the complement regulatory protein CD46 as a physiological inducer of Tr1 cell development. CD46 is a widely expressed transmembrane glycoprotein that inhibits complement activation on host cells[13,14]. CD46 is also used as a receptor by several human pathogens, including measles virus, herpesvirus 6 and two groups of pathogenic bacteria[15–19]. Crosslinking CD46 through its physiological or pathogenic ligands initiates signalling events in several types of human cell[20–23] and in cells derived from CD46 transgenic mice[24].

We stimulated purified human CD4$^+$ lymphocytes with immobilized monoclonal antibodies and assessed their cytokine profile (Fig. 1). Cells stimulated with CD3 and CD46 (CD3/CD46) synthesized small amounts of IL-2, large amounts of IL-10 and intermediate amounts of TGF-β. By contrast, CD3/CD28 activation induced large amounts of IL-2, no IL-10 and barely detectable amounts of TGF-β. Neither stimulatory condition induced IL-4, but both led to the expression of similar quantities of IL-12 and interferon-γ (IFN-γ). Thus, CD3/CD46-stimulated cells have a cytokine profile that is distinct from that induced by stimulation with antibodies to CD3 and CD28 but similar to that of Tr1 cells[5]. IL-12 in the cultures suggests the presence of antigen-presenting

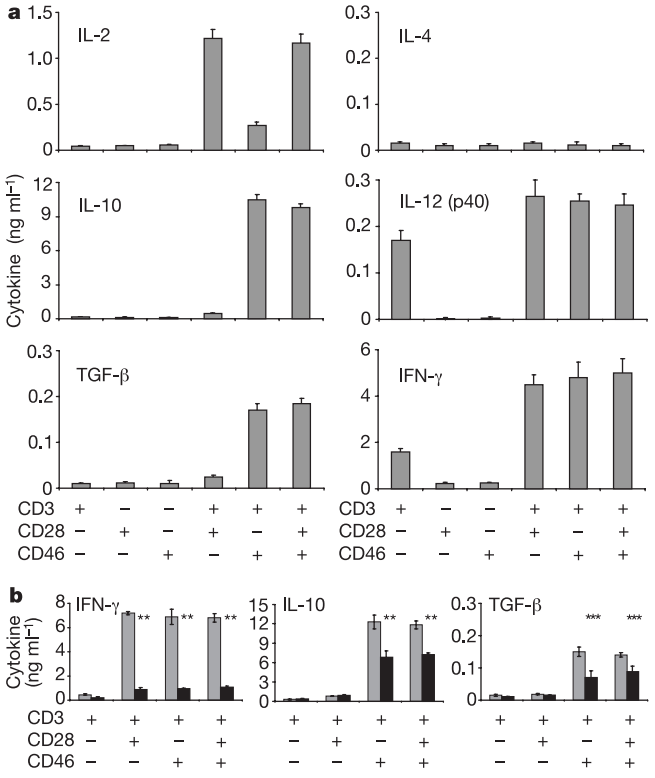

Figure 1 CD3/CD46 stimulation induces IL-10 production in human peripheral blood CD4$^+$ T lymphocytes. **a**, Cytokine profile of stimulated T cells. Supernatants were collected at day 3. The same cytokine profile was observed with a second monoclonal antibody to CD46 (TRA-2-10). **b**, Effect of IL-12 neutralization. Cells were incubated with the indicated monoclonal antibodies in the presence (black bars) or absence (grey bars) of a neutralizing monoclonal antibody to IL-12. In all figures, values shown are the mean ± s.d. of at least three experiments. $^{**}P < 0.005$, $^{***}P < 0.01$.

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cells (APCs). Consistent with this observation, neutralization of this IL-12 completely blocked the synthesis of IFN-γ and reduced IL-10 and TGF-β production by about 40% (Fig. 1b). Thus, CD3/CD46 activation seems to be sufficient for induction of IL-10, but its production can be modulated by factors from other cells.

Purified $CD4^+$ T lymphocytes were sorted for naive ($CD45RA^+$-$CD45RO^-$) and memory ($CD45RA^-CD45RO^+$) cells. Naive and memory T cells constitute 20–40% of the $CD4^+$ population of most donors. We also analysed the cell population with a $CD45RA^+$-$CD45RO^+$ phenotype, because these cells also constitute about a third of peripheral blood $CD4^+$ T cells. These three T-cell subpopulations expressed nearly the same quantities of CD3 and CD46 on their surface, as determined by fluorescence-activated cell sorting (FACS; data not shown). The sorted cell populations were activated in the presence or absence of IL-2 (Fig. 2). Memory $CD4^+$ T cells produced IL-2 on CD3/CD28 activation but did not express IL-10 under any of the activation conditions (data not shown).

By contrast, $CD45RA^+CD45RO^+$ and naive $CD4^+$ T cells both synthesized IL-10 after activation with CD3/CD28/CD46 or CD3/CD46 plus IL-2 (results with naive $CD4^+$ T cells after primary and secondary activation are shown in Supplementary Fig. I). The $CD45RA^+CD45RO^+$ subpopulation (high-responding cells) responded more robustly (Fig. 2a, c) in that they produced IL-10 earlier and in greater quantities (about 3–8-fold more than naive T cells). Neither naive (Supplementary Fig. Ia) nor high-responding (Fig. 2a) cells produced IL-4 or IL-12; however, both cell populations secreted moderate quantities of IFN-γ. Expression of TGF-β was more variable, with small quantities detectable in four out of seven donors. The ability of CD46 to induce IL-10 in isolated $CD8^+$ T cells, natural killer cells, dendritic cells, monocytes or B lymphocytes was also examined. None of these cell populations produced IL-10 after activation with CD3/CD28/CD46 or CD3/CD46 plus IL-2 (data not shown).

The above data established that primary activation of naive or high-responding $CD4^+$ T cells with CD3/CD46 in the presence of IL-2 generates a Tr1 phenotype. To determine whether these cells are then committed to maintain this phenotype, we analysed the properties of these $CD4^+$ T cells on secondary stimulation. Sorted, high-responding $CD4^+$ T cells were initially stimulated for 3 d (Fig. 2b) and subsequently expanded for 6 d in media supplemented with IL-2. The cells were then subjected to secondary stimulation, and IL-10 production was measured after 2 d. High-responding $CD4^+$ T cells, first activated with either CD3/CD28/CD46 or CD3/CD46 plus IL-2, produced large amounts of IL-10 on secondary stimulation (Fig. 2b). Supplementation with IL-2 during secondary stimulation enhanced IL-10 secretion by about 30% (Fig. 2b). The addition of a neutralizing monoclonal antibody to IL-2 abrogated IL-10 production (data not shown), showing that IL-10 production is dependent on IL-2 for both primary and secondary CD46-activated responses.

By contrast, $CD4^+$ T cells initially activated without CD46 crosslinking produced minimal amounts of IL-10 on secondary

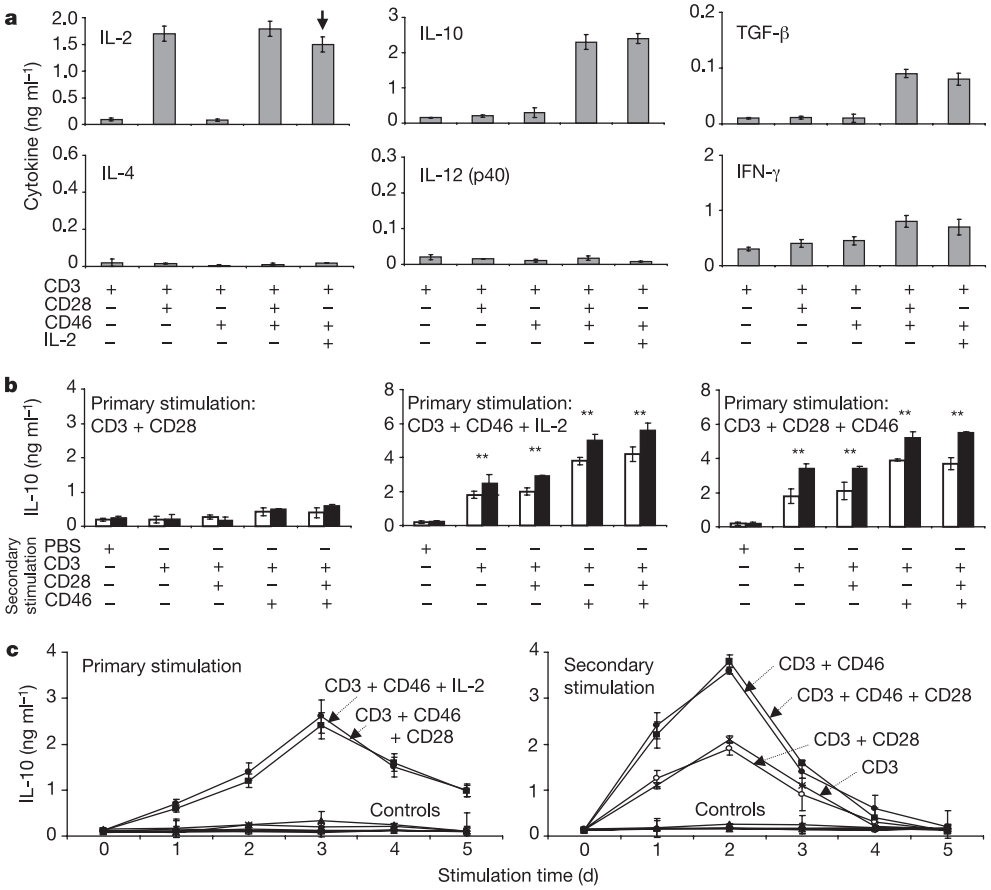

Figure 2 Sorted $CD3^+CD4^+CD45RA^+CD45RO^+$ T cells respond to primary and secondary activation with IL-10 production. **a**, Cytokine profile of primary-stimulated cells. Cells were stimulated for 3 d (with or without IL-2). Arrow indicates the detection of added recombinant IL-2. **b**, Production of IL-10 on secondary stimulation. After primary stimulation for 3 d, cells were expanded and subjected to secondary stimulation in the presence (black bars) or absence of IL-2 (white bars). IL-10 secretion was measured at day 2. **c**, Kinetics of IL-10 production. Left, IL-10 synthesis after primary stimulation. Controls, incubation with monoclonal antibodies to CD3 plus CD28, CD3 plus CD46, CD3 plus isotype control monoclonal antibody, or CD3 plus CD46 plus isotype control. Right, after primary activation with CD3/CD46 plus IL-2, cells were expanded and then subjected to secondary stimulation with the indicated monoclonal antibodies. Controls, incubations with monoclonal antibodies to CD28 or CD46 or with IL-2 alone.

stimulation with CD3/CD46. Therefore, primary stimulation through CD46 is required for IL-10 production in restimulated cells. Notably, with a secondary stimulation, the activation of CD3 alone became sufficient for the induction of IL-10 synthesis (Fig. 2b, c), indicating transition to a memory IL-10-producing phenotype. In addition, IL-10 secretion during secondary activation began and peaked earlier, and was of greater magnitude than that induced by primary activation. Restimulated cells did not produce IL-4 or IL-12, but synthesized small amounts of IL-2 and moderate amounts of IFN-γ (data not shown). In this same set of experiments, naive $CD4^+$ T cells responded in a comparable fashion (Supplementary Fig. I).

Highly purified high-responding or naive $CD4^+$ T cells, after primary activation with CD3/CD46, required the presence of a monoclonal antibody to CD28 or the addition of exogenous IL-2 to produce IL-10. Neutralization of IL-2 abrogated IL-10 production (Fig. 3a). Because CD3/CD46 stimulation is not sufficient to induce production of IL-2, the IL-10 secretion observed in non-sorted $CD4^+$ T cells on stimulation with CD3/CD46 alone (Fig. 1a) is probably due to engagement of CD28 by endogenous B7 expression of APCs in the culture. The primary role of CD28 in the induction of IL-10 may be to provide IL-2.

Because Tr1 cells are characterized by release of IL-10 without concurrent production of IL-2, we examined whether naive and high-responding $CD4^+$ T cells synthesize IL-10 and/or IL-2 by intracellular cytokine staining (Fig. 3b). Cell cultures activated with CD3/CD28/CD46 for 12 h contained mostly non-overlapping populations of IL-10- and IL-2-producing cells. Activation of cells with CD3/CD46 alone did not lead to the production of either cytokine, whereas supplementation with recombinant IL-2 generated only a population of IL-10-expressing cells (Fig. 3b).

CD45RA, CD45RO and CD25 are cell-surface markers that assess the activation state of T cells, specifically their switch from naive ($CD45RA^+CD45RO^-CD25^-$) to memory ($CD45RA^-CD45RO^+$-$CD25^+$) phenotype after CD3/CD28 activation. To determine whether CD3/CD46 activation induces similar changes, we analysed expression of these markers by sorted $CD4^+$ T-cell populations (Fig. 3c, d). Indeed, CD46-activated cells did acquire a memory

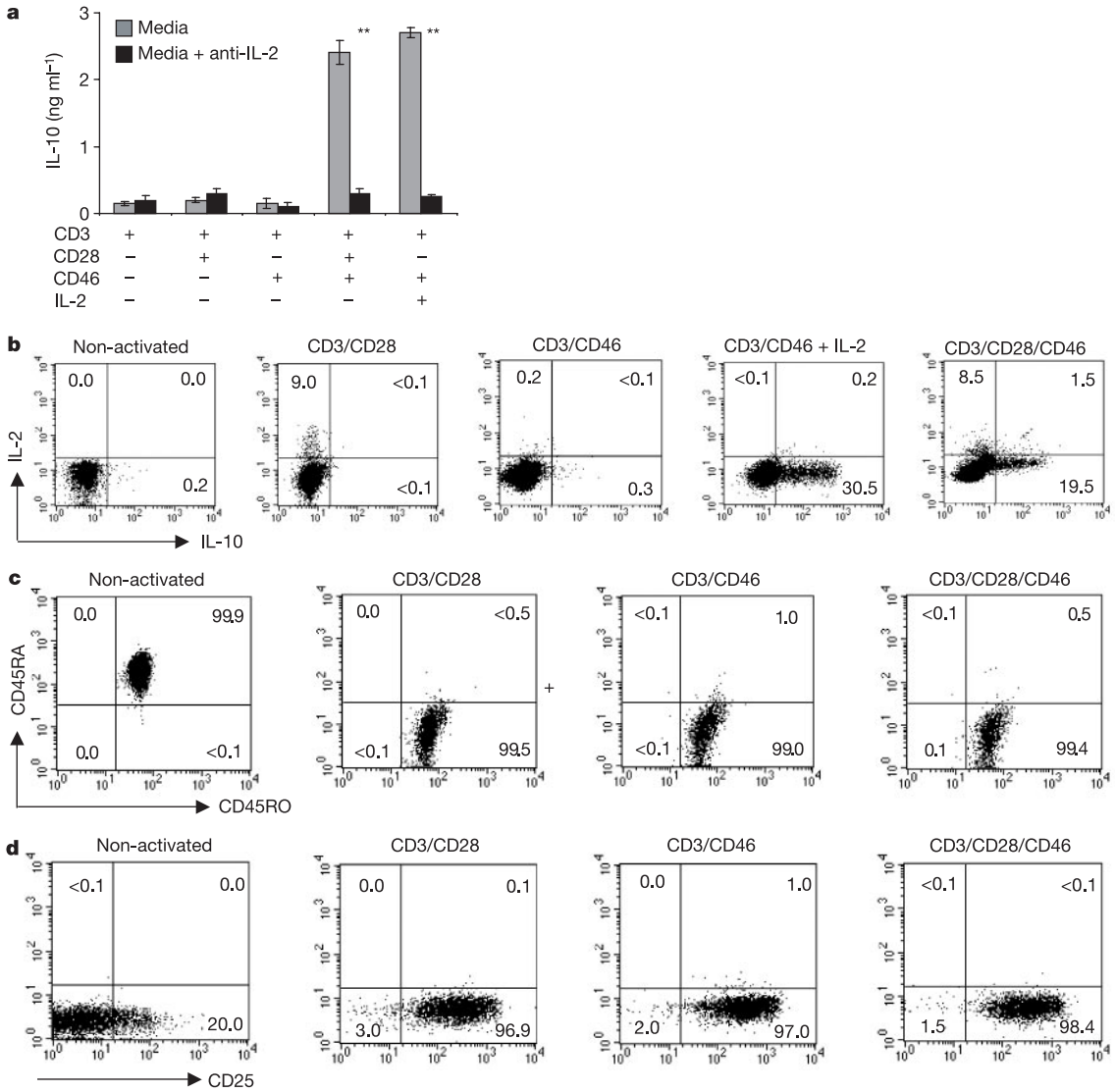

Figure 3 Characteristics of CD3/CD46-activated, sorted $CD3^+CD4^+CD45RA^+CD45RO^+$ T cells. **a**, CD3/CD46-induced IL-10 production is IL-2 dependent. T cells were incubated with the indicated immobilized monoclonal antibodies with or without a monoclonal antibody to IL-2. Supernatants were collected at day 3. **b**, IL-10-producing cells do not express IL-2. T cells were stimulated for 12 h and intracellular cytokine staining was carried out. **c**, CD3/CD46-activated T cells acquire an $CD45RA^-CD45RO^+$ phenotype after primary stimulation. T cells were stimulated with the indicated immobilized monoclonal antibodies. Surface expression of CD45RA and CD45RO was monitored at day 3. **d**, CD3/CD46-activated T cells upregulate CD25 after primary stimulation. T cells were stimulated as indicated for 24 h and expression of CD25 was analysed.

phenotype, because the high-responding, as well as the naive, $CD4^+$ T cells became nearly 100% $CD45RA^-CD45RO^+CD25^+$. This phenotype was not altered after secondary stimulation (data not shown).

We next addressed whether the supernatant from CD3/CD46-activated T cells suppresses proliferation of bystander T cells. At day 3, supernatants from the activated cell populations were transferred to freshly purified $CD4^+$ T cells. These cells were then activated with CD3/CD28 and proliferation was measured at day 7. The supernatants from $CD4^+$ T cells activated by both CD3/CD46 plus IL-2 and CD3/CD28/CD46 inhibited proliferation of bystander $CD4^+$ T cells (Fig. 4a, top). This inhibition was not observed when a neutralizing monoclonal antibody to IL-10 was added (Fig. 4a, bottom). Supernatants derived from sorted, naive $CD4^+$ T cells, from non-sorted $CD4^+$ T cells and from secondary-stimulated $CD4^+$ T cells also inhibited proliferation of bystander T cells (data not shown). As expected, supernatants from CD3/CD28-activated $CD4^+$ T cells did not inhibit proliferation of T cells.

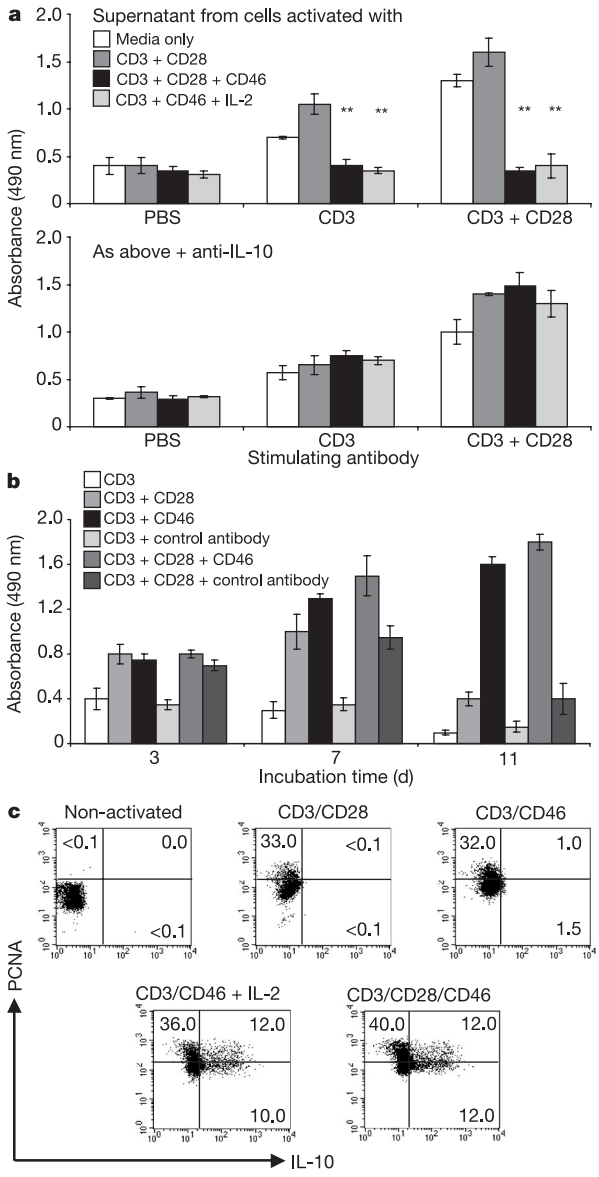

Figure 4 Suppressive and proliferative properties of CD3/CD46-activated $CD4^+$ T cells. **a**, CD3/CD46-stimulated T cells inhibit the proliferation of bystander $CD4^+$ T cells through IL-10. Top, sorted $CD3^+CD4^+CD45RA^+CD45RO^+$ T cells were stimulated for 3 d with the indicated antibodies. Supernatants were transferred to freshly purified $CD4^+$ lymphocytes and the mixture was incubated with the indicated immobilized antibodies. Proliferation was measured at day 7. Bottom, as above but with the addition of a neutralizing monoclonal antibody to IL-10. **b**, CD46 induces a sustained proliferative response. Purified $CD4^+$ T cells were activated with immobilized antibodies and proliferation was measured. **c**, CD3/CD46-stimulated IL-10-producing cells proliferate. Sorted $CD3^+CD4^+CD45RA^+CD45RO^+$ T cells were activated for 12 h with the indicated antibodies and stained for intracellular IL-10 and PCNA.

Tr1 cells, derived *in vitro* by stimulation of human $CD4^+$ cells in the presence of IL-10, proliferate poorly on antigenic stimulation[5,11]. By contrast, activation of CD46 induces IL-10 (as shown above) and, as previously reported[22], is a potent costimulator of $CD4^+$ T-cell proliferation. CD3/CD46-activated $CD4^+$ T cells showed a stronger and more sustained proliferative response as compared with CD3/CD28-activated cells (Fig. 4b). Specifically, CD28/CD3-activated cells ceased proliferating by day 7, whereas CD3/CD46-stimulated cells expanded until at least day 11.

The CD3/CD46-mediated induction of cell proliferation and of IL-10 production might be independently triggered events. To analyse whether IL-10-secreting cells are also proliferating, we stained activated cells intracellularly for IL-10 and for proliferating cell nuclear antigen (PCNA). Roughly 60% of the IL-10-secreting cells proliferated strongly, and this response was comparable to that of non-IL-10-producing, CD3/CD46-activated $CD4^+$ T cells (Fig. 4c). Thus, CD3/CD46-activated cells proliferate strongly despite the presence of high amounts of IL-10, whereas CD3/CD28-activated cells cease proliferation in the presence of IL-10. A possible explanation for this observation might be that CD46 signalling downregulates IL-10 receptor expression. We therefore analysed IL-10 receptor expression of CD3/CD46-activated (IL-10-producing) and CD3/CD28-activated (IL-2-producing) cells. There was no difference in receptor expression (data not shown), suggesting that Tr1 cells evade the inhibitory effects of IL-10 by another mechanism.

To assess whether a physiological ligand of CD46 can also induce IL-10, we activated high-responding $CD4^+$ T cells with CD3, CD28 and C3b dimers (in place of monoclonal antibodies to CD46). Activation with CD3/CD28/C3b dimers resulted in IL-10 secretion comparable to that of CD3/CD28/CD46 (Supplementary Fig. II), thus providing a mechanism for CD46 crosslinking by antigens coated with complement fragments during antigen presentation (Supplementary Fig. III).

In summary, signalling events mediated by CD46 activation induce the development of $CD4^+$ T cells to a Tr1 phenotype. This suggests a role for CD46 in human T-cell regulation and establishes a link between the complement system and adaptive immunity[25–27]. Tr1 cells are essential for maintaining peripheral tolerance and preventing autoimmunity[5,11]. They may also modulate the host's immune response to many pathogens[28]. This putative role of CD46 in regulating cellular immune responses might also explain why several human pathogens have chosen CD46 as their receptor[24]. Our identification of CD46 as a physiological stimulus for Tr1 cell differentiation and proliferation should facilitate further characterization of suppressor cell populations and might provide a therapeutic strategy for generating such cells to ameliorate T-cell-mediated pathology. □

Methods

Media and antibodies

We obtained media and supplements from the tissue-culture facility at Washington University School of Medicine. Cells were maintained in RPMI medium with 10% fetal calf serum and 200 mM L-glutamine in the presence or absence of recombinant human IL-2 (BioSource International). The monoclonal antibody GB24 to human CD46 binds to complement control protein repeats 3 and 4 (ref. 29). The other monoclonal antibody to CD46 that we used, TRA-2-10, recognizes an epitope in the first repeat[29]. The monoclonal antibodies to human CD3 (HIT3a) and CD28 (CD28.2), and the function-neutralizing monoclonal antibodies to human IL-10 (JES3-9D7), IL-2 (MQ1-17H12) and IL-12 (C8.6) were purchased from BD PharMingen. Fluorescein isothiocyanate (FITC)- or

phycoerythrin (PE)-labelled monoclonal antibodies to IL-10, IL-2 and IL-4 (BD PharMingen) and PCNA (Santa Cruz) were used for intracellular staining. We obtained the mouse nonspecific isotype-matched control monoclonal antibody (MOPC 31c, IgG1) from Sigma-Aldrich. The monoclonal antibodies to human CD14 (MoP9), CD16 (3G8), CD8 (Leu-2b), CD19 (4G7) and HLA-DR (L243), used for purifying $CD4^+$ cells, were from Becton Dickinson. Monoclonal antibodies to human CD3 (HIT3a), CD4 (RPA-T4), CD45RA (HI100), CD45RO (UCHL1) and CD25 (2A3), labelled with FITC, PE, allophycocyanin or PerCP, were obtained from Becton Dickinson and used for cell sorting. C3b dimers were generated as described[30].

Purification and sorting of $CD4^+$ lymphocytes

$CD4^+$ lymphocytes were purified from whole blood of healthy human donors by separating the mononuclear cells through Ficoll-Paque Plus (Amersham Pharmacia). This population was then incubated with a mixture of IgG monoclonal antibodies to CD8, CD19, CD14, CD16 and HLA-DR to deplete the $CD8^+$ T cells, B lymphocytes, monocytes/macrophages and natural killer cells and dendritic cells, respectively. After incubation with goat antibodies against mouse IgG coupled to magnetic microbeads (Miltenyi Biotech), $CD4^+$ lymphocytes were obtained by passing the cell mixture over MiniMACS magnetic separation columns (Miltenyi Biotech) and collecting the $CD4^+$ cells in the flow through. For experiments with non-sorted cells, only samples with >97% $CD4^+$ cells were used. To isolate T-cell subpopulations from the enriched purified $CD4^+$ T cells, we incubated cells with combinations of fluorochrome-labelled monoclonal antibodies to CD3, CD4, CD45RA and CD45RO, and then sorted them sterilely using a MoFlo high performance cytometer (Cytomation). Blood from seven donors was used in this study.

Primary and secondary cell stimulation

In vitro primary stimulation was carried out in 96-well culture plates coated with monoclonal antibodies to CD3 ($10\,\mu g\,ml^{-1}$), CD28 ($5\,\mu g\,ml^{-1}$), CD46 ($5\,\mu g\,ml^{-1}$) or a matched IgG1 isotype control antibody ($5\,\mu g\,ml^{-1}$). The wells were washed and purified $CD4^+$ lymphocytes ($1.5–2.0 \times 10^5$ cells per well) were added in 150 µl of culture medium. The plates were centrifuged at 200*g* for 1 min and incubated at 37 °C in 5% CO_2. After 3 d of primary stimulation, cells were washed and expanded for 6 d in media supplemented with 40 units ml^{-1} human IL-2. At day 7, cells were counted and subjected to secondary stimulation under conditions similar to those of the primary activation. We carried out all experiments at least three times and analysed each activation condition in triplicate. Statistical significance was determined using the paired Student's *t*-test.

Proliferation assays

We measured cell proliferation rates using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). This colorimetric assay determines the number of living cells using an MTS tetrazolium compound (Owen's reagent). Initial experiments using this system yielded equivalent results to those obtained by [^{3}H]thymidine incorporation. To analyse which cells among an activated cell population expanded, cells ($1.5–2.0 \times 10^5$ per well) were stimulated with immobilized monoclonal antibodies for 12, 24 or 48 h. Brefeldin A (Sigma-Aldrich) was added for the last 4–8 h of culture. After appropriate surface marker staining, cells were fixed and permeabilized for 10 min with 4% paraformaldehyde and 0.1% saponin (Sigma-Aldrich) in Hank's balanced salt solution and then stained for PCNA. Experiments were done at least three times and each condition was analysed in triplicate.

Cytokine analyses

$CD4^+$ cells ($1.5–2.0 \times 10^5$ per well) were incubated for up to 5 d in 96-well plates coated with monoclonal antibodies. We assessed the secretion of IL-2, IL-4, IFN-γ, IL-10, TGF-β and IL-12 in the supernatants by enzyme-linked immunosorbent assay. IL-4 and IL-12 (p40 and p70) were measured using Biotrak Cellular Communication Assay (Amersham Pharmacia). IL-2, IFN-γ, IL-10 and TGF-β were analysed using EASIA kits (BioSource International). To assess the number of cytokine producing cells within a stimulated cell population, cells ($1.5–2.0 \times 10^5$ cells per well) were activated with immobilized monoclonal antibodies for 12, 24, 48 or 72 h. Brefeldin A was added for the last 4–8 h of culture. After appropriate surface marker staining, cells were stained for intracellular cytokines.

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1. Van Parijs, A. & Abbas, A. K. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* **280,** 243–248 (1998).
2. Kappler, J. W., Roehm, N. & Marrack, P. T cell tolerance by clonal elimination in the thymus. *Cell* **49,** 273–280 (1987).
3. Kisielow, P., Bluthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature* **333,** 742–746 (1988).
4. Sakaguchi, S. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* **101,** 455–458 (2000).
5. Groux, H. *et al.* A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389,** 737–742 (1997).
6. Roncarolo, M. G. & Levings, M. K. The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr. Opin. Immunol.* **12,** 676–683 (2000).
7. LeDouarin, N. *et al.* Evidence for a thymus-dependent form of tolerance that is not based on elimination or anergy of reactive T cells. *Immunol. Rev.* **149,** 35–53 (1996).
8. Shevach, E. M. Regulatory T cells in autoimmunity. *Annu. Rev. Immunol.* **18,** 423–449 (2000).
9. Shevach, E. M. S. M. R., Piccirillo, C. A. & Thornton, A. M. Control of T-cell activation by $CD4^+CD25^+$ suppressor T cells. *Immunol. Rev.* **182,** 58–67 (2001).
10. Chen, Y., Kuchroo, V. K., Inobe, J., Hafler, D. A. & Weiner, H. L. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* **265,** 1237–1240 (1994).
11. Roncarolo, M. G., Bacchetta, R., Bordignon, C., Narula, S. & Levings, M. K. Type 1 regulatory cells. *Immunol. Rev.* **182,** 68–79 (2001).
12. Barrat, F. J. *et al. In vitro* generation of interleukin 10-producing regulatory $CD4^+$ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med.* **195,** 603–616 (2002).
13. Cole, J. L., Housley, G. A. Jr, Dykman, T. R., MacDermott, R. P. & Atkinson, J. P. Identification of an additional class of C3-binding membrane proteins of human peripheral blood leukocytes and cell lines. *Proc. Natl Acad. Sci. USA* **82,** 859–863 (1985).
14. Liszewski, M. K., Post, T. W. & Atkinson, J. P. Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. *Annu. Rev. Immunol.* **9,** 431–455 (1991).
15. Dorig, R. E., Marcil, A., Chopra, A. & Richardson, C. D. The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* **75,** 295–305 (1993).
16. Naniche, D. *et al.* Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J. Virol.* **67,** 6025–6032 (1993).
17. Santoro, F. *et al.* CD46 is a cellular receptor for human herpes virus 6. *Cell* **99,** 817–827 (1999).
18. Okada, N., Liszewski, M. K., Atkinson, J. P. & Caparon, M. Membrane cofactor protein (MCP; CD46) is a keratinocyte receptor for the M protein of group A streptococcus. *Proc. Natl Acad. Sci. USA* **92,** 2489–2493 (1995).
19. Kallstrom, H., Liszewski, M. K., Atkinson, J. P. & Jonsson, A.-B. Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*. *Mol. Microbiol.* **25,** 639–647 (1997).
20. Karp, C. L. *et al.* Mechanism of suppression of cell-mediated immunity by measles virus. *Science* **273,** 228–231 (1996).
21. Kallstrom, H., Islam, M. S., Berggren, P. O. & Jonsson, A. B. Cell signaling by the type IV pili of pathogenic *Neisseria*. *J. Biol. Chem.* **273,** 21777–21782 (1998).
22. Astier, A., Trescol-Biemont, M.-C., Azocar, O., Lamouille, B. & Rabourdin-Combe, C. Cutting edge: CD46, a new costimulatory molecular for T cells, that induces p120CBL and LAT phosphorylation. *J. Immunol.* **164,** 6091–6095 (2000).
23. Wang, G., Liszewski, M. K., Chan, A. C. & Atkinson, J. P. Membrane cofactor protein (MCP; CD46): isoform-specific tyrosine phosphorylation. *J. Immunol.* **164,** 1839–1846 (2000).
24. Marie, J. *et al.* Linking innate and acquired immunity: divergent role of CD46 cytoplasmic domains in T cell-induced inflammation. *Nature Immunol.* **3,** 659–666 (2002).
25. Nielsen, C. H., Fischer, E. M. & Leslie, R. G. The role of complement in the acquired immune response. *Immunology* **100,** 4–12 (2000).
26. Dempsey, P. W., Allison, M. E., Akkaraju, S., Goodnow, C. C. & Fearon, D. T. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science* **271,** 348–350 (1996).
27. Prodeus, A. P. *et al.* A critical role for complement in maintenance of self-tolerance. *Immunity* **9,** 721–731 (1998).
28. McGuirk, P. & Mills, K. H. G. Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends. Immunol.* **23,** 450–455 (2002).
29. Liszewski, M. K. *et al.* Dissecting sites important for complement regulatory activity in membrane cofactor protein (MCP; CD46). *J. Biol. Chem.* **275,** 37692–37701 (2001).
30. Krych-Goldberg, M. *et al.* Decay accelerating activity of complement receptor type 1 (CD35). *J. Biol. Chem.* **274,** 31160–31168 (1999).

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