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Monocyte:T cell interaction regulates human T cell activation through a CD28/CD46 crosstalk

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Abstract

T cell activation requires engagement of the T cell receptor and of at least one costimulatory molecule. The key role of CD28 in inducing T cell activation has been reported several decades ago and the molecular mechanisms involved well described. The complement regulator CD46 also acts as a costimulatory molecule for T cells but, in contrast to CD28, has the ability to drive T cell differentiation from producing some IFNγ to secreting some potent anti-inflammatory IL-10, acquiring a so-called Type I regulatory phenotype (Tr1). Proteolytic cleavage of CD46 occurs upon costimulation and is important for T cell activation and IL-10 production. The observation that CD46 cleavage was reduced when PBMC were costimulated compared to purified naive T cells led us to hypothesize that interactions between different cell types within the PBMC were able to modulate the CD46 pathway. We show that CD46 downregulation is also reduced when $CD4^+$ T cells are co-cultured with autologous monocytes. Indeed, monocyte:T cell co-cultures impaired CD46–mediated T cell differentiation and coactivation, by reducing downregulation of surface CD46, lowering induction of the early activation marker CD69, as well as reducing the levels of IL-10 secretion. Blocking of CD86 could partly restore CD69 expression and cytokine secretion, demonstrating that the CD28-CD86 pathway regulates CD46 activation. Direct concomitant ligation of CD28 and CD46 on $CD4^+$ T cells also modulated CD46 expression and regulated cytokine production. These data identify a crosstalk between two main costimulatory pathways and provide novel insights into the regulation of human T cell activation.

Introduction

T cell activation results from a complex integration of signals conjointly received by the TCR and additional costimulatory molecules. It involves, at least, a two-signal stimulation process. Signal 1 requires TCR ligation, which ensures the antigen specificity of the response and signal 2, or the costimulatory signal, is required to fully activate T cells. Further signals such as cytokines or alternate surface receptor also contribute to T cell

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activation. CD28, a member of the B7 family, is the main costimulatory molecule [1, 2]. However, additional costimulatory molecules fulfilling the role of signal 2 have been described, although their specific role hasn't been fully elucidated. Among these, CD46 was initially identified as a regulator for complement activity, binding to C3b and C4b complement and promoting their cleavage by factor I, hence protecting the cells from complement damage [3, 4]. CD46 also binds to several pathogens [5-7]. A role in the adaptive immune response was also later described, as costimulation with CD3/CD46 led to increased T cell proliferation [8, 9], induced morphological changes [10], affected T cell polarity [11] and, importantly, promoted a switch from Th1 to Tr1 Treg differentiation on addition of IL-2 [12]. This was characterized by secretion of high amounts of IL-10 [12] and granzyme B [13]. This regulatory pathway is altered in a number of chronic inflammatory diseases such as multiple sclerosis, rheumatoid arthritis and asthma, as IL-10 production upon CD46 costimulation is impaired [14-19]. This underlines the importance of the pathway for correct immune homeostasis. Importantly, CD46 expression is different between mice and men as CD46 is confined to the testis in mice while being ubiquitously expressed by human cells. Expression of human CD46 in transgenic mice however showed that CD46 has immunoregulatory properties in these mice [9].

CD46 is a type I membrane protein expressed by all human nucleated cells. CD46 ectodomain is composed of four short consensus repeats and a region rich in serine, threonine and proline. This is followed by a transmembrane segment and a short cytoplasmic tail. Due to alternative splicing, multiple isoforms are produced, that include two distinct intracytoplasmic tails [20]. *In vitro* studies using primary human T cells showed the antagonistic effects of CD46 cytoplasmic tails in T cell activation and cytokine production [21, 22]. These studies also illustrated the importance of CD46 processing for its function. Activation of CD46 on primary T cells led to its enzymatic processing, its ectodomain being partly cleaved by matrix-metalloproteinase (MMP), followed by the cleavage of its two cytoplasmic tails. Notably, inhibition of MMPs led to decreased IL-10 production by CD46 costimulated T cells. Moreover, CD46 tail processing allows T cell activation but also T cell termination, and therefore is key to ensure T cell homeostasis [21, 22]. Expression of CD46 at the surface of T cells is furthermore regulated by a variety of mediators, such as vitamin D [23] and prostaglandin E2 [24]. Together, these data underline the importance of the regulation of CD46 expression on activated T cells for T cell function.

Herein, we first report the differential levels of CD46 downregulation on activated PBMC versus purified $CD4^+$ T cells, which could be reproduced by co-culture of purified monocytes and T cells. By dissecting the mechanisms involved, we have identified that CD28 controlled the CD46 pathway in activated human T cells, which notably modulated secretion of IL-10. Overall, we demonstrate a crosstalk between CD28 and CD46 that regulates CD46 expression and function, providing novel insights on the regulation of human T cell activation.

Results

Reduced downregulation of CD46 on costimulated T cells in the presence of monocytes

CD46 is enzymatically processed upon CD3/CD46 costimulation. Notably, CD3/CD46 costimulation induces the MMP-dependent shedding of its ectodomain, leading to a strong downregulation of CD46 surface expression [21]. In our previously published data, most of the experiments were conducted using highly purified $CD4^+$ T cells. We therefore first compared levels of CD46 downregulation in cultured purified $CD4^+$ T cells (>95% purity) and PBMC. PBMC were isolated from blood and $CD4^+$ T cells purified from PBMC, keeping some PBMC for comparison. Cells were then activated *in vitro* by immobilized anti-CD3 and anti-CD3/CD46 antibodies for 2 days and CD46 expression assessed. As expected, a significant downregulation of CD46 expression was observed on costimulated $CD4^+$ T cells. However, CD46 downregulation was much reduced on the lymphocyte gate when PBMC were analyzed (Figure 1a and 1b). As monocytes constitute the main population in PBMC susceptible to interact with T cells, we hypothesized that the difference in CD46 expression between purified T cells and PBMC might be due to the interaction between monocytes and T cells. We therefore isolated $CD14^+$ monocytes as well as autologous $CD4^+$ T cells from PBMC (Figure 1c), and compared CD46 expression on costimulated T cells activated in the presence or absence of purified monocytes (Figure 1d). As expected, there was significant CD46 downregulation in purified $CD4^+$ T cells (top panel), but also on monocytes activated by anti-CD46, suggesting that shedding of CD46 also occurs in monocytes. In contrast, we observed that co-culture of monocytes and T cells significantly reduced CD46 downregulation, albeit only in $CD4^+$ T cells. Similar data were obtained when we gated T cells using an anti-CD3 that specifically stained T cells and not the monocytes, or when we gated out the monocytes using an anti-HLA-DR or CD14 marker that did not stain T cells (Figure e and not shown). These data indicate that the co-culture of monocytes and T cells modulates CD46 expression on activated T cells.

Co-culture of $CD4^+$ T cells and monocytes modulates T cell phenotype

We next determined whether co-culture with monocytes also affected the phenotype of these T cells. We assessed expression of the two markers of activation, CD69 and CD25, after 2 or 5 days of culture (Figure 2). As expected, a strong upregulation of CD69 and CD25 was detected after CD3/CD46 costimulation of $CD4^+$ T cells. Although T cells co-cultured with monocytes were activated and expressed high levels of CD25, they did not sustain CD69 expression after 5 days of culture, compared to purified T cells. As expected, an increase in CD69 and CD25 expression was detected for T cells activated by CD3 in the presence of monocytes. Therefore, these data indicate that co-culture of purified T cells with monocytes cells did not affect T cell activation as CD25 expression was enhanced, but that it partially impaired CD46 downregulation and costimulated T cells did not sustain expression of CD69.

Co-culture of monocytes and T cells affects cytokine production

One of the main outcomes of CD46 costimulation for human T cells is their switch from producing IFNγ to secreting IL-10, in the presence of IL-2 [12]. Thus, CD46 costimulation can promote a regulatory phenotype by increasing the IL-10:IFNγ ratio [16], and CD46 processing

is an important step in the release of IL-10 [21]. As we observed a lower downregulation of CD46 expression in the co-culture conditions, we hypothesized that it would affect production of these two cytokines, and assessed their levels. $CD4^+$ T cells were activated by anti-CD3 or anti-CD3/CD46 in the presence or absence of monocytes and the levels of IL-10 and IFNγ were determined by ELISA after 4 days (Figure 3a). Co-culture conditions led to an increase in IFNγ production. As expected, a higher IL-10:IFNγ ratio was obtained for CD46-costimulated T cells compared to CD3-activated T cells (Figure 3b). However, this ratio was significantly lowered in the presence of monocytes, and addition of monocytes also reduced this ratio for CD3-activated T cells. Cytokines are mainly produced by the proliferating T cells after 5 days of culture, and we did not detect any significant levels of cytokines in the supernatants of monocytes cultured alone (not shown). However, as monocytes can produce some IL-10, we could not exclude that monocytes secreted some cytokines in the co-culture conditions. Hence, we also assessed cytokine production by secretion assays on $CD3^+$ gated T cells. As expected, CD46 costimulation of CD4+ T cells enhanced IL-10 secretion compared to CD3 activation. Co-culture with monocytes strongly decreased IL-10 secretion and increased IFNγ production by activated T cells (Figure 3c). Proliferation was also examined by staining the $CD4^+$ T cells with eFluor® 670 before activation in the presence or absence of monocytes. While addition of monocytes promoted proliferation of T cells activated by CD3, there was no significant effect on proliferation of costimulated T cells (Figure 3d), suggesting that the shift in cytokine production towards IFNγ detected by ELISA was not correlated to increased cell numbers for CD46-costimulated T cells.

The CD28:CD86 pathway is involved in modulating CD46 function in the co-culture experiments

We next hypothesized that monocytes were modulating T cells through their cognate CD86:CD28 interaction. CD28 activation on T cells is mediated by binding to CD80 (B7-1)/CD86 (B7-2) expressed on antigen presenting cells. As unstimulated human monocytes do not express CD80 [25], we therefore assessed the effect of blocking CD86 using a blocking antibody, or an IgG1 control. We also determined whether CD80 was induced by *in vitro* culture in our model. However, CD80 expression on the monocytes could not be detected, even after 3 days of co-culture (data not shown). Addition of a blocking anti-CD86 Ab increased expression of CD69 on co-cultured T cells (Figure 4a), and increased IL-10 production by costimulated T cells in the presence of monocytes (Figure 4b). As CD46 is key to control IL-10 production by activated T cells, these data suggest that CD28 may impact the CD46 pathway through its interaction with CD86 to modulate CD46 function in T cells.

CD28 costimulation modulates CD46 surface expression on activated T cells

To confirm the existence of a crosstalk between CD28 and CD46 on T cells, we next determined whether CD28 concomitant co-stimulation affected the levels of CD46 on costimulated T cells. Purified human $CD4^+$ T cells were left unstimulated or were activated with anti-CD3, anti-CD3/anti-CD28, anti-CD3/anti-CD46 or anti-CD3/anti-CD28/anti-CD46. CD46 surface expression was examined by flow cytometry after 2 days (Figure 5a). As previously shown, CD3/CD46 costimulation induced a strong downregulation of CD46

surface expression. Concomitant coactivation by CD28 led to an enhanced residual expression of CD46, compared to cells costimulated by CD3/CD46 alone. A similar effect of CD28 was observed after 2 and 4 days (not shown) and similar effects were also observed when another CD46 antibody was used (E4.3 clone, not shown). These data demonstrate that the CD28 pathway controls CD46 expression on activated T cells. We also observed that CD46 costimulation enhanced CD28 expression on T cells, but that concomitant CD28 and CD46 activation reduces this CD28 induction (not shown). Altogether, this demonstrates a crosstalk between CD28 and CD46 that regulates their expression on activated T cells.

Lastly, to confirm the importance of CD28 ligation for the modulation of CD46 function, we compared cytokine production and proliferation of purified T cells after activation by anti-CD3/CD28, anti-CD3/CD46 or anti-CD3/CD46/CD28 (Figure 5b-e). Costimulation of T cells with either CD28 or CD46 or both led to enhanced IL-10 secretion compared to CD3 activation alone. Secretion of IFNγ was mainly induced when CD28 was co-engaged and, as previously reported, low levels of IFNγ were detected in CD46-costimulated T cell supernatants in absence of CD28 costimulation. However, concomitant coactivation significantly enhanced IFNγ production. When the IL-10:IFNγ ratio was calculated, as expected, there was an increased IL-10 ratio upon CD46 costimulation, but this ratio was significantly decreased when CD28 was concomitantly activated, although still higher than upon engagement of CD28 alone. In contrast, we did not find any significant effect of CD28 on the CD46-mediated proliferation. These data show that engagement of CD28 and CD46 alters the response of the T cells compared to single coactivation, as CD28/CD46 costimulation, by controlling secretion of IL-10 and IFNγ, promotes regulatory function compared to CD28 alone but decreases it when compared to CD46 activation alone.

Inhibition of MMPs has a stronger effect on CD28-CD46 costimulated T cells than CD46 costimulated T cells

CD46 is partially shed by MMPs upon CD46 costimulation and MMP cleavage is necessary for IL-10 production [21, 22]. Hence, we next determined whether addition of a broad MMP inhibitor could restore CD46 surface expression of CD28/CD46 costimulated T cells (Figure 6). MMP inhibition partly decreased CD46 shedding on CD46 costimulated T cells, as previously shown, but had a stronger effect on T cells concomitantly activated by CD28, indicating that CD28 effect on CD46 was principally mediated by MMPs.

Discussion

T cells are at the heart of the immune response and their activation is precisely controlled by a variety of signals. Besides engagement of the TCR by the antigenic peptide, ligation of several surface receptors dictate the fate of the T cells, by controlling their proliferation and differentiation. Most studies have focused on one particular costimulatory or co-inhibitory receptor. Herein, we have identified a crosstalk between two of the main costimulatory molecules for human T cells, CD28 and CD46. CD46 activation on human T cells leads to its proteolytic cleavage that is important for IL-10 secretion [21]. Our data first show that the co-culture of monocytes and T cells modulates the CD46 pathway, by lowering CD46 downregulation and reducing IL-10 secretion while enhancing IFNγ production. A crosstalk

between monocytes and human γδ T cells has been previously reported [26]. Moreover, a recent study has shown that interaction of activated monocytes with $CD25^+Foxp3^+$ regulatory T cells could modulate their cytokine production upon CD3 activation, by increasing production of both pro-inflammatory IFNγ and IL-17 and of anti-inflammatory IL-10, and they remained suppressive [27]. Our study shows that resting monocytes have the ability to modulate T cell activation, including the CD46 pathway, although it is possible that monocytes undergo a slight differentiation after plating them into culture wells. It was previously published that human monocytes can upregulate CD80 during *in vitro* cultures [25]. However, Dewar *et al* reported that monocytes cultured for 5 days in the presence of M-CSF or GM-CSF did not express CD80 [28], and we were not able to detect CD80 in our system. By using a blocking antibody, we show that the interaction between CD86 and CD28 is at least partially responsible for this effect, although it is likely that other interactions are involved as well. Moreover, direct antibody ligation of CD28 also down-modulates the regulatory functions of CD46, by reducing its downregulation and promoting IFNγ production. Regulation of the MMP/ADAM involved in cleaving CD46 ectodomain may therefore be regulated by CD28. It is therefore possible that CD28 stimulation alters levels of MMPs/ADAM involved in the shedding of CD46, which is supported by the fact that MMP inhibition further enhanced CD46 expression on CD28/CD46 costimulated T cells.

Although CD46 was initially described as a complement regulator, it has become apparent that it also modulates the adaptive immune response, by inducing T cell activation and differentiation, as well as influencing inflammation and modulating T cell polarity [8-10, 12, 29]. The importance of CD46 function in T cells has been further evidenced by its plasticity in the regulation of T cell activation and/or differentiation, and overall T cell homeostasis [16, 21, 29, 30]. Most importantly, it is now clear that CD46 costimulation on primary $CD4^+$ T cells induces the secretion of the anti-inflammatory cytokine IL-10, while lowering IFNγ production [12, 16]. Strikingly, this regulatory pathway is dysregulated in a number of pathologies (MS, RA and asthma), which again underlines the key role of CD46 in the control of inflammation [15-19]. Hence, the understanding of the mechanisms regulating CD46 expression and function in primary human T cells is key to fully comprehend how to regulate inflammation. The identification of the CD28/CD46 crosstalk may furthermore shed light on the defective CD46 pathway observed in MS, as increased expression of CD28 has been observed on MS T cells [31] and, therefore, this increased CD28 expression may reduce CD46 regulatory functions in MS T cells.

The question of the ligands involved in this context is therefore critical, in other words, what engages CD46 in an APC:T cell interaction. CD3-activated T cells release some C3b, an endogenous ligand for CD46 [16, 32]. Therefore this likely provokes the necessary stimulus required to stimulate CD46. Moreover, this may also explain some of the observed effects on CD3-activated T cells. Finally, the local C3b produced may also feedback to the monocytes, and we have observed that CD46 ligation of monocytes could affect their phenotype (unpublished data). Timing of the different activation pathways may also be important for the T cell in deciding whether to favor activation and / or regulation. It has been previously reported that CD28 was critical for initial T cell expansion, while 4-1BB had a later effect in the response being essential for the survival and/or responsiveness of memory CD8 T cells [33] and this differential effect was due to a switch in costimulation from CD28 to

4.1BB [34]. Therefore, similar timing engagement of CD28 versus CD46 in human T cell activation may occur for human T cells. Naïve T cells are likely first activated by CD3/CD28, producing some C3b subsequently engaging CD46, which may switch the response to regulation. Lastly, memory T cells that have increased CD46 expression compared to naive $CD4^+$ T cells (unpublished data) require less costimulation than naïve T cells, suggesting that CD46 may play a preponderant role in memory T cells. Of note, we have also co-cultured monocytes with naïve T cells, and similar trends on T cell activation were observed (not shown). Moreover, CD46 could have a key role in tolerance, and targeting the CD46 pathway in peptide induced tolerance (PIT) [35] may prove to be a beneficial approach, further enhancing tolerance through regulation of IL-10 secretion. Targeting of CD46 could be achieved by using recombinant proteins derived from adenovirus Ad35, as recently reported [36].

Altogether, the existence of a crosstalk between CD28 and CD46 highlights the complexity of the control of human T cell activation, and understanding the molecular mechanisms involved in T cell plasticity may provide novel therapeutics approach to restore immune homeostasis.

Methods

Cell purification and activation

PBMC were isolated by Ficoll-Hypaque density gradient centrifugation (Pharmacia LKB Biotechnology, Piscataway, NJ), from citrated (0.38%) venous blood from healthy donors obtained after informed consent, and ethical approval from the NHS Lothian Board, and conformed to the provisions of the Declaration of Helsinki. $CD4^+$ T cells were negatively isolated using magnetic beads (StemCell, purification > 97%), Monocytes were purified by $CD14^+$ positive selection (StemCell, purifity >90%). Cells were cultured in RPMI 1640 with 10% fetal calf serum at 1×10^6 per well in 48-well plate pre-coated with anti-CD3 (OKT3, 5μg/ml), anti-CD46 (MC120.6, 10 μg/ml kindly provided by Dr. Chantal Rabourdin-Combe, recognizing the SCR1 domain of CD46) or anti-CD28 (28.2, Ebiosciences, 5μg/ml). Activated primary T cells also received rhIL-2 (Life Technologies - 10U/ml). Co-cultures were performed by adding 0.2 million of monocytes followed by 0.2 million of T cells per well. For the blocking experiments, a blocking anti-CD86 (LEAF Purified anti-CD86, clone IT 2.2, 10 μg/ml, Biolegend) or irrelevant IgG1 (LEAF Purified IgG1, clone MODC21, 10 μg/ml) was added to the culture.

Cytokine detection

Cell culture supernatants from the 48-well plates (as described in the cell activation section) were collected after five days of stimulation, and both IL-10 and IFNγ secretion was determined by ELISA specific for human IL-10 (BD Pharmingen, San Diego, CA) and IFNγ (Endogen, Rockford, IL). Alternatively, cytokine secretion was also determined using the IL-10 and IFNγ secretion assays from Miltenyi using anti-IFNγ-FITC and anti-IL-10-PE detection reagents, according to the manufacturer's instructions and cells were gated on $CD3\text{-}APC^+$ T cells. Gates were done using the unstimulated T cells and were also verified using unstained activated T cells.

Flow cytometry

The expression level of CD46, CD69 and CD25 was assessed by flow cytometry, by incubating the cells with the antibodies at 4°C for 20 min in FACS buffer (PBS containing 1% fetal calf serum). We used anti-CD46-FITC (clone MEM-258 – Biolegend, recognizing the SCR4 domain of CD46, hence a different domain that the stimulating antibody), anti-CD69-FITC (Biolegend) and anti-CD25-APC (Biolegend). In some instances, we also stained the cells with CD3-APC or CD28-PE, or CD14-APC to gate the cells. Samples were run with a FACSCalibur and data analyzed using FlowJo. Relative expression to staining with the control was calculated by calculating the ΔMFI (MFI obtained with antibody - MFI obtained with isotype control).

Proliferation assay

Proliferation was determined by pre-labeling purified T cells with eFluor 670 cell proliferation stain (eBioscience) before activation following the manufacturer's instructions, and assessing remaining fluorescence after 4 days. In some experiments, $CD4^+$ T cells were plated in duplicate at 5×10^4 in a pre-coated 96 well-plate as described above. After three days stimulation at 37°C, cells were pulsed with 1μCi of [^{3}H]thymidine (Amersham) and cultured for 18hrs. Proliferation was then determined using a Liquid Scintillation Counter (Wallac, Boston, USA).

Statistics

The groups were analyzed using Graphpad Prism software. Flow cytometry data were analyzed using the Wilcoxon test, when assessing paired samples. ELISA data are the average of duplicate wells, and the average obtained for the different donors were analyzed using the Wilcoxon test. All p-values are two-tailed and with a 95% confidence interval.

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References

1. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol. 2002; 2:116–26. [PubMed: 11910893]
2. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunol Rev. 2009; 229:12–26. [PubMed: 19426212]
3. Seya T, Turner JR, Atkinson JP. Purification and characterization of a membrane protein (gp45-70) that is a cofactor for cleavage of C3b and C4b. J Exp Med. 1986; 163:837–55. [PubMed: 3950547]
4. Lublin DM, Liszewski MK, Post TW, Arce MA, Le Beau MM, Rebentisch MB, et al. Molecular cloning and chromosomal localization of human membrane cofactor protein (MCP). Evidence for inclusion in the multigene family of complement-regulatory proteins. J Exp Med. 1988; 168:181–94. [PubMed: 3260937]
5. Cattaneo R. Four viruses, two bacteria, and one receptor: membrane cofactor protein (CD46) as pathogens' magnet. J Virol. 2004; 78:4385–8. [PubMed: 15078919]
6. Russell S. CD46: a complement regulator and pathogen receptor that mediates links between innate and acquired immune function. Tissue Antigens. 2004; 64:111–8. [PubMed: 15245366]

7. Price JD, Schaumburg J, Sandin C, Atkinson JP, Lindahl G, Kemper C. Induction of a regulatory phenotype in human CD4+ T cells by streptococcal M protein. J Immunol. 2005; 175:677–84. [PubMed: 16002662]
8. Astier A, Trescol-Biemont MC, Azocar O, Lamouille B, Rabourdin-Combe C. Cutting edge: CD46, a new costimulatory molecule for T cells, that induces p120CBL and LAT phosphorylation. J Immunol. 2000; 164:6091–5. [PubMed: 10843656]
9. Marie JC, Astier AL, Rivailler P, Rabourdin-Combe C, Wild TF, Horvat B. Linking innate and acquired immunity: divergent role of CD46 cytoplasmic domains in T cell induced inflammation. Nat Immunol. 2002; 3:659–66. [PubMed: 12055630]
10. Zaffran Y, Destaing O, Roux A, Ory S, Nheu T, Jurdic P, et al. CD46/CD3 costimulation induces morphological changes of human T cells and activation of Vav, Rac, and extracellular signal-regulated kinase mitogen-activated protein kinase. J Immunol. 2001; 167:6780–5. [PubMed: 11739493]
11. Oliaro J, Pasam A, Waterhouse NJ, Browne KA, Ludford-Menting MJ, Trapani JA, et al. Ligation of the cell surface receptor, CD46, alters T cell polarity and response to antigen presentation. Proc Natl Acad Sci U S A. 2006
12. Kemper C, Chan AC, Green JM, Brett KA, Murphy KM, Atkinson JP. Activation of human CD4(+) cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature. 2003; 421:388–92. [PubMed: 12540904]
13. Grossman WJ, Verbsky JW, Tollefsen BL, Kemper C, Atkinson JP, Ley TJ. Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. Blood. 2004; 104:2840–8. [PubMed: 15238416]
14. Astier AL. T-cell regulation by CD46 and its relevance in multiple sclerosis. Immunology. 2008; 124:149–54. [PubMed: 18384356]
15. Astier AL, Meiffren G, Freeman S, Hafler DA. Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. J Clin Invest. 2006; 116:3252–7. [PubMed: 17099776]
16. Cardone J, Le Friec G, Vantourout P, Roberts A, Fuchs A, Jackson I, et al. Complement regulator CD46 temporally regulates cytokine production by conventional and unconventional T cells. Nat Immunol. 2010; 11:862–71. [PubMed: 20694009]
17. Ma A, Xiong Z, Hu Y, Qi S, Song L, Dun H, et al. Dysfunction of IL-10-producing type 1 regulatory T cells and CD4+CD25+ regulatory T cells in a mimic model of human multiple sclerosis in Cynomolgus monkeys. Int Immunopharmacol. 2009; 9:599–608. [PubMed: 19539557]
18. Martinez-Forero I, Garcia-Munoz R, Martinez-Pasamar S, Inoges S, Lopez-Diaz de Cerio A, Palacios R, et al. IL-10 suppressor activity and ex vivo Tr1 cell function are impaired in multiple sclerosis. Eur J Immunol. 2008; 38:576–86. [PubMed: 18200504]
19. Xu YQ, Gao YD, Yang J, Guo W. A defect of CD4+CD25+ regulatory T cells in inducing interleukin-10 production from CD4+ T cells under CD46 costimulation in asthma patients. J Asthma. 2010; 47:367–73. [PubMed: 20528588]
20. Russell SM, Loveland BE, Johnstone RW, Thorley BR, McKenzie IF. Functional characterisation of alternatively spliced CD46 cytoplasmic tails. Transplant Proc. 1992; 24:2329–30. [PubMed: 1413082]
21. Ni Choileain S, Weyand NJ, Neumann C, Thomas J, So M, Astier AL. The dynamic processing of CD46 intracellular domains provides a molecular rheostat for T cell activation. PLoS One. 2011; 6:e16287. [PubMed: 21283821]
22. Ni Choileain S, Astier AL. CD46 processing: a means of expression. Immunobiology. 2012; 217:169–75. [PubMed: 21742405]
23. Kickler K, Ni Choileain S, Williams A, Richards A, Astier AL. Calcitriol modulates the CD46 pathway in T cells. PLoS One. 2012; 7:e48486. [PubMed: 23144765]
24. Kickler K, Maltby K, Ni Choileain S, Stephen J, Wright S, Hafler DA, et al. Prostaglandin E2 affects T cell responses through modulation of CD46 expression. J Immunol. 2012; 188:5303–10. [PubMed: 22544928]
25. Fleischer J, Soeth E, Reiling N, Grage-Griebenow E, Flad HD, Ernst M. Differential expression and function of CD80 (B7-1) and CD86 (B7-2) on human peripheral blood monocytes. Immunology. 1996; 89:592–8. [PubMed: 9014827]

26. Eberl M, Roberts GW, Meuter S, Williams JD, Topley N, Moser B. A rapid crosstalk of human gammadelta T cells and monocytes drives the acute inflammation in bacterial infections. PLoS Pathog. 2009; 5:e1000308. [PubMed: 19229322]
27. Walter GJ, Evans HG, Menon B, Gullick NJ, Kirkham BW, Cope AP, et al. Interaction with activated monocytes enhances cytokine expression and suppressive activity of human CD4+CD45ro+CD25+CD127(low) regulatory T cells. Arthritis Rheum. 2013; 65:627–38. [PubMed: 23280063]
28. Dewar AL, Doherty KV, Hughes TP, Lyons AB. Imatinib inhibits the functional capacity of cultured human monocytes. Immunol Cell Biol. 2005; 83:48–56. [PubMed: 15661041]
29. Oliaro J, Pasam A, Waterhouse NJ, Browne KA, Ludford-Menting MJ, Trapani JA, et al. Ligation of the cell surface receptor, CD46, alters T cell polarity and response to antigen presentation. Proc Natl Acad Sci U S A. 2006; 103:18685–90. [PubMed: 17116876]
30. Ludford-Menting MJ, Crimeen-Irwin B, Oliaro J, Pasam A, Williamson D, Pedersen N, et al. The Reorientation of T-Cell Polarity and Inhibition of Immunological Synapse Formation by CD46 Involves Its Recruitment to Lipid Rafts. J Lipids. 2011; 2011:521863. [PubMed: 21490803]
31. Fransson ME, Liljenfeldt LS, Fagius J, Totterman TH, Loskog AS. The T-cell pool is anergized in patients with multiple sclerosis in remission. Immunology. 2009; 126:92–101. [PubMed: 18624727]
32. Liszewski MK, Kolev M, Le Friec G, Leung M, Bertram PG, Fara AF, et al. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. Immunity. 2013; 39:1143–57. [PubMed: 24315997]
33. Bertram EM, Lau P, Watts TH. Temporal segregation of 4-1BB versus CD28-mediated costimulation: 4-1BB ligand influences T cell numbers late in the primary response and regulates the size of the T cell memory response following influenza infection. J Immunol. 2002; 168:3777–85. [PubMed: 11937529]
34. Bertram EM, Dawicki W, Sedgmen B, Bramson JL, Lynch DH, Watts TH. A switch in costimulation from CD28 to 4-1BB during primary versus secondary CD8 T cell response to influenza in vivo. J Immunol. 2004; 172:981–8. [PubMed: 14707071]
35. Mackenzie KJ, Nowakowska DJ, Leech MD, McFarlane AJ, Wilson C, Fitch PM, et al. Effector and central memory T helper 2 cells respond differently to peptide immunotherapy. Proc Natl Acad Sci U S A. 2014; 111:E784–93. [PubMed: 24516158]
36. Hay J, Carter D, Lieber A, Astier AL. Recombinant Ad35 adenoviral proteins as potent modulators of human T cell activation. Immunology. 2014

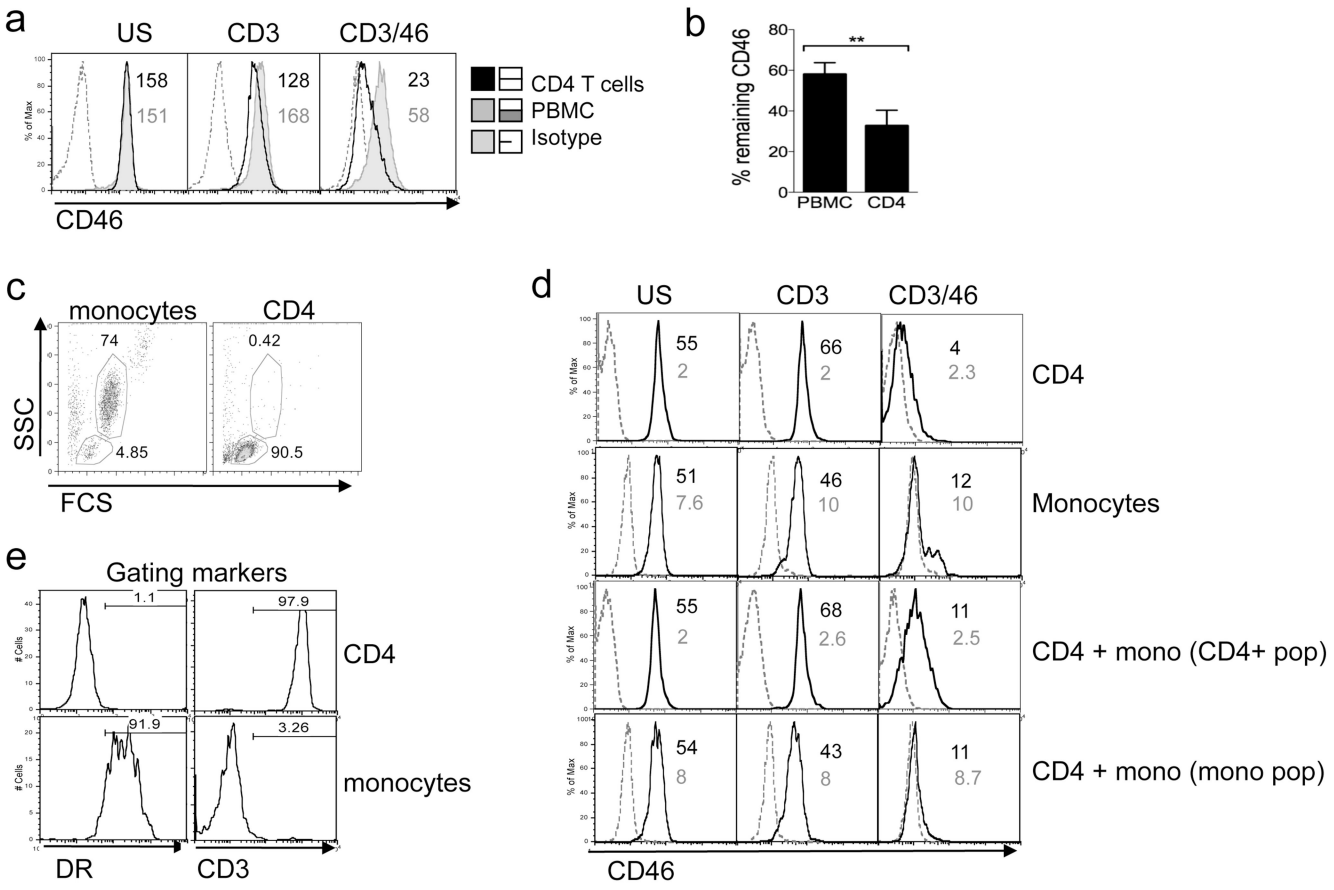

Figure 1. Reduced downregulation of CD46 on T cells in activated PBMCs and CD4$^+$ T cell:monocyte co-cultures compared to purified CD4$^+$ T cells

(a) PBMC (tinted grey histograms) or purified CD4$^+$ T cells (black histograms) were left unstimulated (US) or were activated by immobilized anti-CD3 and anti-CD46 for 2 days and CD46 expression analyzed by flow cytometry. The MFI of the stained samples are indicated for CD4+ T cells (in black) and PBMC (in grey). Dashed line = isotype control. Representative plots shown. **(b**) The percentage of remaining surface CD46 was calculated for CD46-costimulated PBMC and CD4$^+$ T cells (n=5, mean +/− SEM). **(c)** CD4$^+$ T cells and CD14+ monocytes were purified and co-cultured and **(d)** expression of CD46 was determined after 2 days. The MFI obtained for CD46 (in black) and for the isotype (in grey) are indicated. Representative of 3 donors. Staining using DR and CD3 is shown in **(e).**

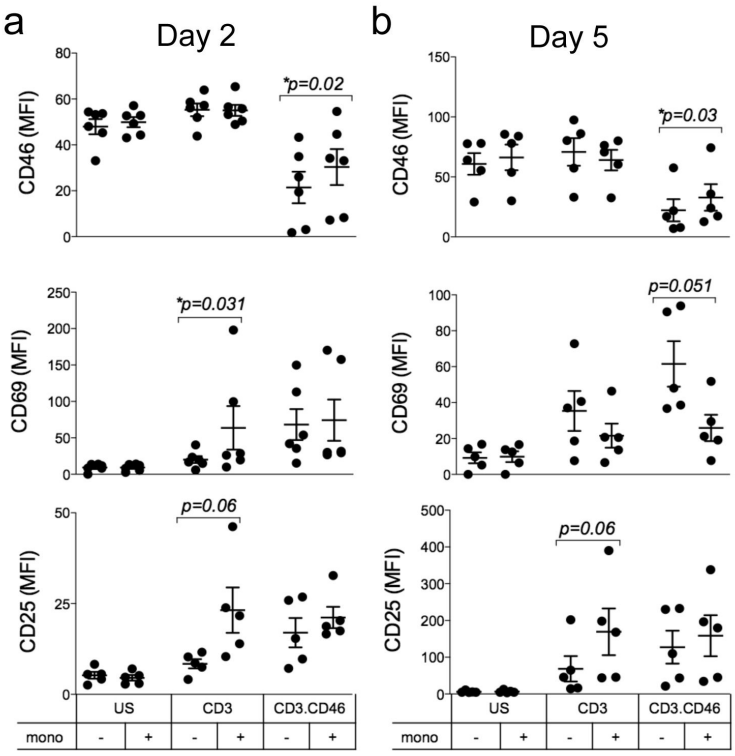

Figure 2. CD4$^+$ T cell:monocyte co-cultures modulate T cell phenotype

Purified CD4$^+$ T cells cultured alone or in the presence of autologous monocytes were left unstimulated (US) or were activated by immobilized anti-CD3 and anti-CD46 for **(a)** 2 or **(b)** 5 days, and expression of CD46, CD69 and CD25 was determined by flow cytometry on the lymphocyte population gated by size (mean +/− SEM).

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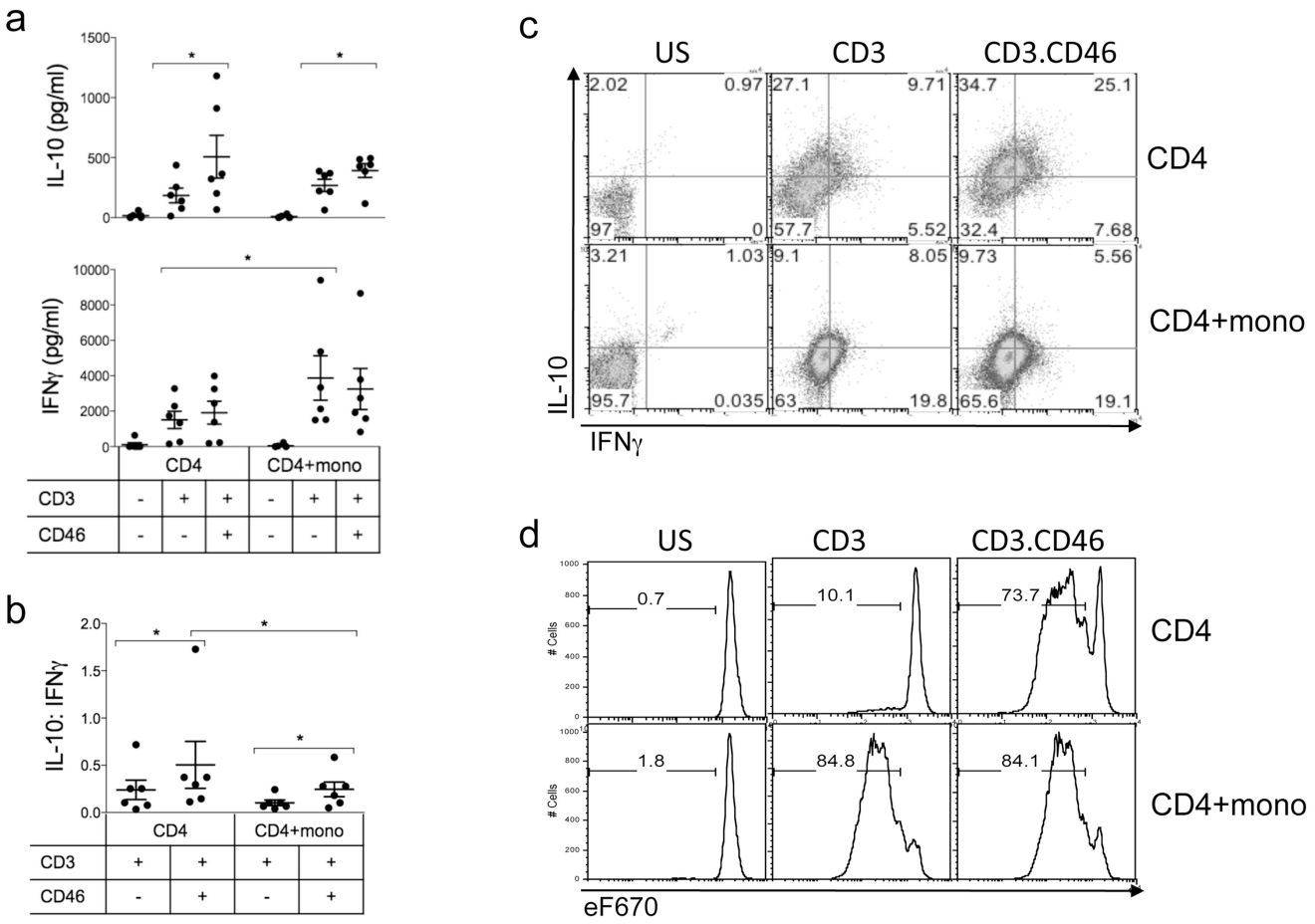

Figure 3. CD4$^+$ T cell:monocyte co-cultures modulate IL-10 and IFNγ production by activated T cells

(a) The production of IL-10 and IFNγ in the culture supernatants of activated purified T cells or CD4$^+$ T cells:monocyte co-cultures at day 4 was assessed by ELISA (n=5; mean +/− SEM). **(b)** The IL-10:IFNγ ratio is also represented. **(c)** IL-10 and IFNγ secretion of CD3$^+$ gated T cells cultured in the presence or absence of monocytes was assessed by secretion assays. **(d)** Purified CD4$^+$ T cells were pre-labeled with eFluor 670 before activation by immobilized anti-CD3 or anti-CD3/anti-CD46 in the presence or absence of monocytes. Four days later, proliferation was monitored by flow cytometry.

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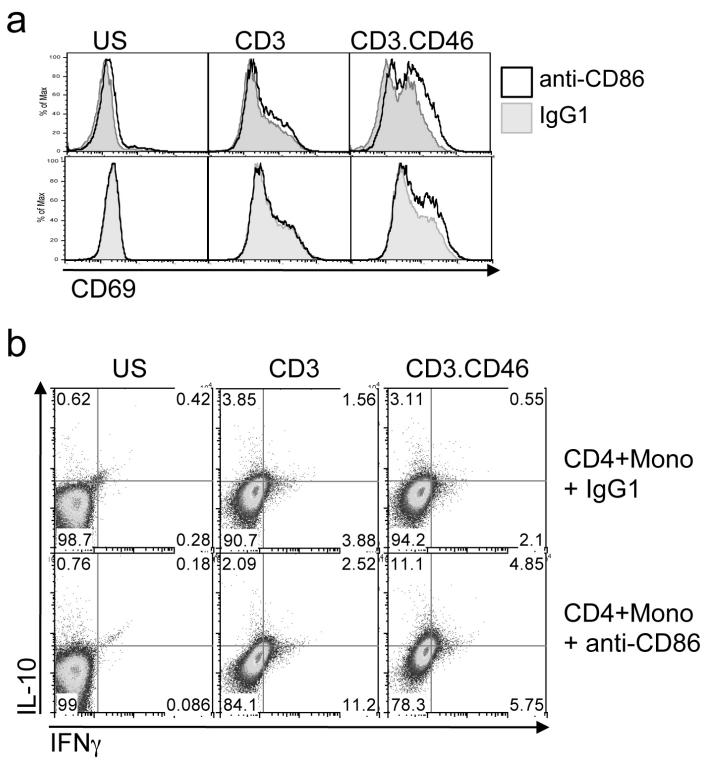

Figure 4. Blocking CD86 in CD4$^+$ T cell:monocyte cocultures enhances CD69 expression and IL-10 production by activated T cells

(a) CD4$^+$ T cells were activated with autologous monocytes by immobilized anti-CD3 and/or anti-CD46 in the presence of a blocking anti-CD86 antibody (black histograms) or irrelevant IgG1 control (tinted grey histograms). CD69 surface expression was then monitored by flow cytometry. 2 donors are shown (out of n=4). **(b)** Production of IL-10 and IFNγ were assessed after 4 days by secretion assays.

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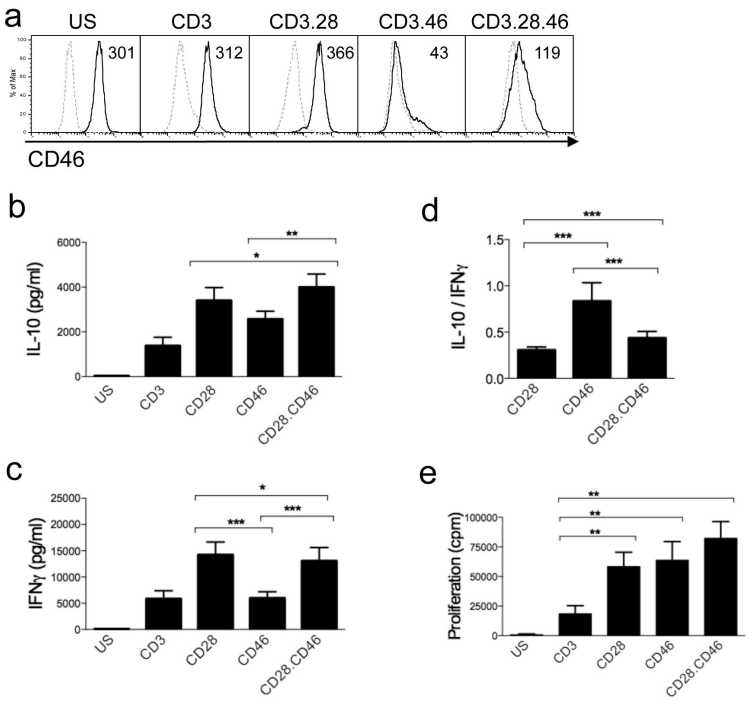

Figure 5. Concomitant CD28 and CD46 ligations modulate cytokine production

Purified $CD4^+$ T cells were left unstimulated (US), or were activated by immobilized anti-CD3 alone or in addition of anti-CD28 and/or anti-CD46 antibodies, as indicated. **(a)** Expression of CD46 was determined after 2 days. Dashed line = isotype control. The MFI obtained for CD46 are indicated. The production of IL-10 **(b)** and IFNγ **(c)** in the culture supernatants of activated purified T cells was determined by ELISA (n=14). **(d)** The IL-10:IFNγ ratio is also represented for costimulated T cells. **(e)** Proliferation was monitored by thymidine incorporation. Mean +/− SEM is represented.

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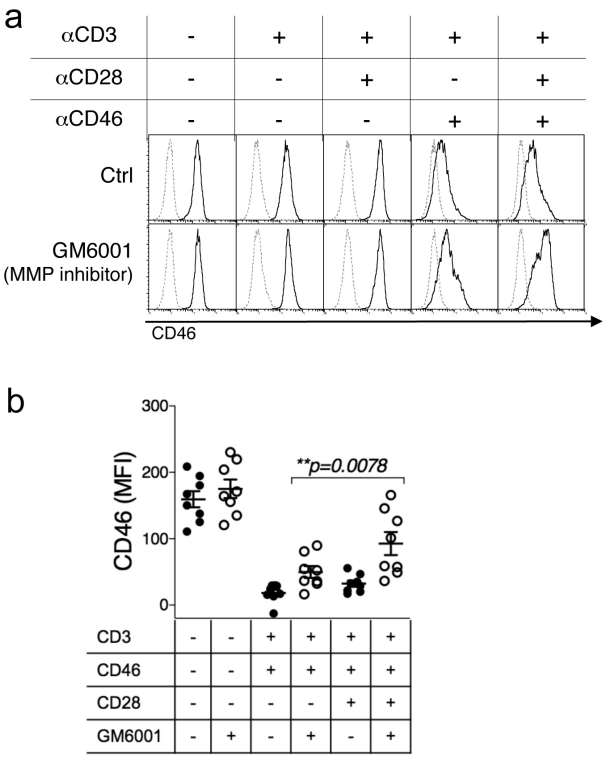

Figure 6. MMP inhibition results into increased surface CD46 expression on CD28/CD46 costimulated T cells

$CD4^+$ T cells were activated with immobilized antibodies as indicated in presence or absence of GM6001, a broad metalloproteinase inhibitor (10 μM). After 2 days, the cell surface expression of CD46 was determined by flow cytometry. Dashed line = isotype control. **(a)** shows the representative plots obtained for one donor, and **(b)** shows the data obtained for the different donors (n=8; mean +/− SEM).

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