Glucocorticoid-Induced TNF Receptor Family-Related Protein Ligand is Requisite for Optimal Functioning of Regulatory CD4+ T Cells

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.3389/fimmu.2014.00035

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:11879918

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Glucocorticoid-induced tumor necrosis factor receptor family-related protein ligand is requisite for optimal functioning of regulatory CD4+ T cells

Gongxian Liao1*, Michael S. O’Keeffe1, Guoxing Wang1, Boaz van Driel1, Rene de Waal Malefy2, Hans-Christian Reinecker3, Roland W. Herzog4 and Cox Terhorst1*

1 Division of Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
2 Biologics Discovery, Merck Research Laboratories, Palo Alto, CA, USA
3 Gastrointestinal Unit and Center for the Study of Inflammatory Bowel Disease, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
4 Department of Pediatrics, University of Florida, Gainesville, FL, USA

INTRODUCTION

CD4+ CD25+ FoxP3+ regulatory T cells (Treg), which develop in the thymus or can be induced in peripheral organs, control many aspects of the immune response (1–6). Tregs constitutively express glucocorticoid-induced tumor necrosis factor receptor family-related protein ligand (GITR-L), which is inducible in effector T cells. This in turn induces GITR-L, a soluble form of the natural ligand of GITR, we found recently that glucocorticoid-induced tumor necrosis factor receptor family-related protein ligand (GITR-L), which is expressed by antigen presenting cells, on the development and expansion of Tregs. We found that GITR-L is dispensable for the development of naturally occurring FoxP3+ Treg cells in the thymus. However, the expansion of Treg in GITR-L−/− mice is impaired after injection of the dendritic cells (DCs) inducing factor Flt3 ligand. Furthermore, DCs from the liver of GITR-L−/− mice were less efficient in inducing proliferation of antigen-specific Treg cells in vitro than the same cells from WT littermates. Upon gene transfer of ovalbumin into hepatocytes of GITR-L−/− FoxP3(GFP) reporter mice using adenovirus-associated virus (AAV8-OVA) the number of antigen-specific Treg in liver and spleen is reduced. The reduced number of Tregs resulted in an increase in the number of ovalbumin-specific CD8+ T effector cells. This is highly significant because proliferation of antigen-specific CD8+ cells itself is dependent on the presence of GITR-L, as shown by in vitro experiments and by adoptive transfers into GITR-L−/− Rag2−/− and Rag2−/− mice that had received AAV8-OVA. Surprisingly, administering αCD3 significantly reduced the numbers of FoxP3+ Treg cells in the liver and spleen of GITR-L−/− but not WT mice. Because soluble Fc-GITR-L partially rescues αCD3 induced in vivo depletion of the CD103+ subset of FoxP3+ CD4+ Treg cells, we conclude that expression of GITR-L by antigen presenting cells is requisite for optimal Treg-mediated suppression of immune responses including those in response during gene transfer.

Keywords: GITR-L, TNFRSF18, Flt3L, Treg, CX3CR1

Glucocorticoid-induced tumor necrosis factor receptor family-related protein (TNFRSF18, CD357) is constitutively expressed on regulatory T cells (Tregs) and is inducible in effector T cells. In this report, we examine the role of glucocorticoid-induced TNF receptor family-related protein ligand (GITR-L), which is expressed by antigen presenting cells, on the development and expansion of Tregs. We found that GITR-L is dispensable for the development of naturally occurring FoxP3+ Treg cells in the thymus. However, the expansion of Treg in GITR-L−/− mice is impaired after injection of the dendritic cells (DCs) inducing factor Flt3 ligand. Furthermore, DCs from the liver of GITR-L−/− mice were less efficient in inducing proliferation of antigen-specific Treg cells in vitro than the same cells from WT littermates. Upon gene transfer of ovalbumin into hepatocytes of GITR-L−/− FoxP3(GFP) reporter mice using adenovirus-associated virus (AAV8-OVA) the number of antigen-specific Treg in liver and spleen is reduced. The reduced number of Tregs resulted in an increase in the number of ovalbumin-specific CD8+ T effector cells. This is highly significant because proliferation of antigen-specific CD8+ cells itself is dependent on the presence of GITR-L, as shown by in vitro experiments and by adoptive transfers into GITR-L−/− Rag2−/− and Rag2−/− mice that had received AAV8-OVA. Surprisingly, administering αCD3 significantly reduced the numbers of FoxP3+ Treg cells in the liver and spleen of GITR-L−/− but not WT mice. Because soluble Fc-GITR-L partially rescues αCD3 induced in vitro depletion of the CD103+ subset of FoxP3+ CD4+ Treg cells, we conclude that expression of GITR-L by antigen presenting cells is requisite for optimal Treg-mediated suppression of immune responses including those in response during gene transfer.

Keywords: GITR-L, TNFRSF18, Flt3L, Treg, CX3CR1

Edited by: Federico Mingozzi, UPMC Paris 6, France; Genethon, France
Reviewed by: Federico Mingozzi, UPMC Paris 6, France; Genethon, France
David William Scott, Uniformed Services University of the Health Sciences, USA

*Correspondence: Gongxian Liao, Division of Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, 3 Blackfan Circle, CLS-908, Boston, MA 02115, USA
E-mail: gian@bidmc.harvard.edu; Cox Terhorst, Division of Immunology, Beth Israel Deaconess Medical Center, Harvard Medical School, 3 Blackfan Circle, CLS-928, Boston, MA 02115, USA
E-mail: cterhorst@bidmc.harvard.edu

www.frontiersin.org
February 2014 | Volume 5 | Article 35 | 1
AA V8-OVA mediated expression of foreign protein in hepatocytes

AAV8-OVA vector (containing an ovalbumin expression cassette driven by AAV-EF1α) was packaged into serotype 8 capsid as described previously (16). Vector was injected i.v. into FoxP3(−/−) and GITR-L−/−FoxP3(−/−) mice at a dose of 1010 vector genome/mouse. Five weeks later, leukocytes from liver, spleen, and thymus were stained with TCRvα2.

Results

Also, Ly6G NK1.1+ cells FACS sorted from the liver of Cx3CR1(−/−) mice 7 days after AAV8-OVA injection were incubated with OT-II CD4+ or CFSE-labeled OT-1 CD8+ T cells for 3 days. OT-II CD4+ T cell cultures were stained with TCRvα2 and FoxP3. OT-1 CD8+ T cell culture was stained with TCRvα2 and proliferating CD8+ cells were evaluated by CFSE dilution.

In vitro activation of CD4+ T cells

CD4+ T cells from the spleen of FoxP3(−/−) mice were negatively selected using a CD4+ T cells isolation kit (Miltenyi, Auburn, CA, USA) and were activated with αCD3-coupled microbeads in a round bottom 96-well plate in the presence or absence of Flt3L-Fc fusion protein (10 ng/mouse/injection) for 2 days as described previously (9). Cells were stained with CD4 and CD103. Expression of FoxP3 was judged by reporter gene EGFP. Cell numbers were counted with a Countess Automated Cell Counter (Invitrogen, Grand Island, NY, USA).

Isolation of liver leukocytes

Liver leukocytes were isolated as described previously (27). Briefly, liver was washed and filtered through a 70 μm cell strainer. Hepatocytes and cell debris were removed by spinning at 300 rpm for 10 min. Supernatant was centrifuged at 1500 rpm for 10 min to collect cells. Leukocytes were isolated from the interface of a 40 and 70% Percoll gradient.

Statistical analysis used Prism 4.0c software (GraphPad, San Diego, CA, USA). Statistical comparisons were performed using the two-tailed Student’s t-test. Values of P < 0.05 were considered to be statistically significant.

Materials and methods

Mice

B6, OT-II Tg, and CX3CR1(GFP) reporter mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). OT-I × Rag−/− mice were purchased from Taconic Labs (Germantown, NY, USA). GITR-L−/− and FoxP3-ires-EGFP-SV40 knock-in [FoxP3(GFP)] B6 mice were described previously (8, 25). GITR-L−/− mice were crossed with FoxP3(−/−) and CX3CR1(GFP) mice to generate GIXR-L−/−FoxP3(−/−) and GIXR-L−/−CX3CR1(GFP) B6 mice. All animals were housed in the Center for Life Science animal facility of BIDMC. The Guide for the Care and Use of Laboratory Animals was followed in the conduct of the animal studies of the Institutional Animal Care and Use Committee at BIDMC. Veterinary care was given to any animals requiring medical attention.

Antibodies

Anti-CD11b-PacBlu, αCD11b-FITC, αCD4-PE, αCD4-APC, αCD11c-APC, αCD11c-PE, αTCRvα2-PE, and αCD3s(145–2C11) were purchased from BioLegend (San Diego, CA, USA). Anti-Ly6C-PerCP and αFoxP3-APC were products of eBioscience (San Jose, CA, USA). Anti-Ly6G-PE, αNK1.1-PE, αCD8α-PacBlu, αCD25-PE, and αCD103-Alexa Fluor 647 were products from BD Biosciences (San Jose, CA, USA). Flt3L-Fc fusion protein was purchased from BioXCell (West Lebanon, NH, USA). Anti-IL-2 was purchased from R&D Systems (Minneapolis, MN, USA). Fc-GITR-L fusion protein was produced as described previously (9).

In vivo activation of dendritic cells and TREG with Flt3L

Flt3L-Fc fusion protein (10 ng/mouse/injection) was i.p. injected into FoxP3(−/−) and GIXR-L−/−FoxP3(−/−) mice for nine consecutive days as described previously (26). Leukocytes from the spleen and liver were analyzed at day 10.

Cellularity in mice after αCD3-mediated activation of T cells by in vivo

Anti-CD3e was i.p. injected into Cx3CR1(−/−) and GIXR-L−/−CX3CR1(−/−) mice (20 μg/mouse, one injection). After 72 h, leukocytes of the spleen and liver were stained with CD4 and FoxP3. CX3CR1+ cells were evaluated by expression of the reporter gene GFP.

Isolation of liver leukocytes

Liver leukocytes were isolated as described previously (27). Briefly, liver was washed and filtered through a 70 μm cell strainer. Hepatocytes and cell debris were removed by spinning at 300 rpm for 10 min. Supernatant was centrifuged at 1500 rpm for 10 min to collect cells. Leukocytes were isolated from the interface of a 40 and 70% Percoll gradient.

Statistical analysis used Prism 4.0c software (GraphPad, San Diego, CA, USA). Statistical comparisons were performed using the two-tailed Student’s t-test. Values of P < 0.05 were considered to be statistically significant.

Results

Flt3L-induced expansion of Treg was impaired in GITR-L deficient mice due to a partially reduced number of dendritic cell subpopulations

We previously found that after administering a Fc-GITR-L fusion protein to WT mice the number of Treg cells increased, which was confirmed by studies with GITR-L transgenic mice (9–11, 28). Surprisingly, we found that GITR-L was dispensable for the development of naturally occurring Treg, as the number of FoxP3+ Treg cells was normal in the thymus and spleen of GIXR-L−/−FoxP3(−/−) mice under resting conditions (Figure 1A; Figure S1 in Supplementary Material).
After the injection of Fc-Flt3L fusion protein, both the numbers and frequency of CD11c−/− DCs in the liver of FoxP3(GFP) and GITR-L−/− FoxP3(GFP) mice were significantly increased in the spleen and liver. This Fc-Flt3L-induced expansion was, however, significantly reduced in GITR-L−/− FoxP3(GFP) mice (Figures 1B,C). The total number of CD4+ T cells in the spleen was also lower in GITR-L−/− FoxP3(GFP) mice than the WT counterparts (Figure 1D). Thus, GITR-L plays a significant role in the expansion of Treg in the peripheral tissues.

We next evaluated whether the impaired Flt3L-induced expansion of Treg cells in GITR-L−/− FoxP3(GFP) mice correlated with reduced numbers of DCs and macrophages (MØ) (31, 32). As shown in Figure 2A and Figure S2A in Supplementary Material, the percentage of CD11c+CD11b+ and CD11c−CD11b− DCs was reduced in the spleen of GITR-L−/− FoxP3(GFP) mice as compared to FoxP3(GFP) mice. Although the number of conventional CD11c+ DCs in the liver was normal (Figure 2A), the percentage of pDCs in GITR-L−/− FoxP3(GFP) mice was higher than that of their WT counterparts (Figure 2B; Figure S2B in Supplementary Material and Data not shown). The frequency of CD11c−CD11b+ MØ was comparable between these two mice (Figure 2C). Taken together, these data indicate that after Flt3L induction, GITR-L affects the expansion and differentiation of subpopulations of DCs, which in turn leads to expansion of Tregs.

FIGURE 1 | Flt3L-induced expansion of Treg. Flt3L-Fc fusion protein was injected into FoxP3(GFP) and GITR-L−/− FoxP3(GFP) mice (10 ng/mouse/ injection, nine injections). CD4+CD8− T cells from the thymus, spleen, and/or liver were analyzed by FACS for FoxP3 expression based on the expression of reporter protein EGFP. (A) Percentages of CD4+FoxP3+ Treg in the thymus and spleen of FoxP3(GFP) and GITR-L−/− FoxP3(GFP) mice without stimulation. Percentages (B) and representative staining (C) of Treg in the spleen and liver after administering Flt3L. (D) Number of CD4+ T cells in the spleen and liver of FoxP3(GFP) and GITR-L−/− FoxP3(GFP) mice after administering Flt3L. Filled circle represents FoxP3(GFP) mouse. Open circle represents GITR-L−/− FoxP3(GFP) mouse. Each circle represents one mouse.

FIGURE 2 | CD11c+ DCs after Flt3L induction. Flt3L-Fc fusion protein was injected into FoxP3(GFP) and GITR-L−/− FoxP3(GFP) mice as described in Figure 1. Different subsets of myeloid cells in the spleen and liver were analyzed. (A) Percentages of CD11c+ cells in the spleen and liver. (B) Percentages of CD11c+PDCA1+ cells in the spleen and liver. (C) Percentages of CD11c−CD11b+ cells. Filled circle represents FoxP3(GFP) mouse. Open circle represents GITR-L−/− FoxP3(GFP) mouse. Each circle represents one mouse.

Material and Data not shown). The frequency of CD11c−CD11b+ MØ was comparable between these two mice (Figure 2C). Taken together, these data indicate that after Flt3L induction, GITR-L affects the expansion and differentiation of subpopulations of DCs, which in turn leads to expansion of Tregs.

GITR-L−/− CX3CR1+ DCs ISOLATED FROM THE LIVER ARE LESS EFFICIENT THAN WT CX3CR1+ DCs IN THE IN VITRO INDUCTION OF OVA-SPECIFIC TREG AND CD8+ T CELLS

To directly test whether the absence of GITR-L in DC subpopulations affects proliferation of antigen-specific GITR+ Treg and CD8+ cells, we immunized GITR-L−/− CX3CR1(GFP) and WT CX3CR1(GFP) mice by gene transfer with AAV8-OVA (Figure 3A). One week after injection of AAV8-OVA, liver CX3CR1(GFP)+ cells purified by FACS were incubated with OVA-specific OT-II CD4+ T cells or OT-I CD8+ cells for 3 days. GITR-L−/− CX3CR1+ cells were less efficient in inducing Treg as compared to the same cells isolated from WT mice (Figures 3B,C). Since activated CD8+ cells carry GITR on their surface, we also evaluated whether in vitro proliferation of CD8+ T cells would be affected by the absence of GITR-L from the surface of these DCs. Indeed, the proliferation of CD8+ OT-I cells was reduced when cocultured with liver CX3CR1+ cells from AAV8-OVA-primed GITR-L−/− CX3CR1(GFP) mice compared to OT-I cells cultured with WT CX3CR1+ DCs (Figures 3D,E).

We conclude that GITR-L on the surface of antigen presenting cells can drive proliferation of both FoxP3+CD4+ Treg cells and activated CD8+ T cells in an antigen-specific manner.
AFTER AAV8-ova GENE TRANSFER, THE NUMBER OF ANTIGEN-SPECIFIC TREG IN GITR-L-/- FoxP3 MICE IS REDUCED, WHICH RESULTS IN AN INCREASED NUMBER OF OVA-SPECIFIC CD8+ T CELLS

Because targeted expression of exogenous protein in hepatocytes by AAV8-mediated gene transfer induces a Treg-mediated tolerance (16), we assessed whether this process involves GITR-L. To assess this, we injected an AAV8-OVA vector into FoxP3(GFP) and GITR-L-/- FoxP3(GFP) mice and determined the number of OVA-specific Treg and CD8+ T cells. Consistent with the results when administering Flt3L, there was a reduced percentage of OVA-specific FoxP3+ TCRv2+ T cells in the spleen and liver of GITR-L-/-FoxP3(GFP) mice as compared to that of WT mice 5 weeks after vector administration (Figure 4A). Conversely, AAV-mediated OVA expression in the hepatocytes induced an increased percentage of OVA-specific CD8+ TCRv2+ T cells in the spleen and liver of GITR-L-/-FoxP3(GFP) mice (Figure 4B). By contrast, the total cell numbers were comparable between these two mouse strains (Figure 4C). The data suggest that GITR-L deficiency may impair the induction of antigen-specific Tregs (16–18, 21, 33), which may at least partially compromise their immunosuppressive capability.

As the in vitro data suggest that GITR-L expression on DCs causes the expansion of CD8+ T cells, this in vivo result might underestimate the consequences of the reduced number of the Tregs in the GITR-L-/- mice. To test whether GITR-L is implicated in the in vivo expansion of antigen-specific CD8+ T cells, we used a system in which the Treg-mediated suppression is absent. To this end, we injected AAV8-OVA into Rag-/- and GITR-L-/- Rag-/- mice followed by the adoptive transfer of OT-I CD8+ T cells after 1 week (Figure 5A). Eight weeks after transfer of OT-I CD8+ T cells, the number of CD8+ T cells in the blood of the GITR-L-/- Rag-/- recipients was significantly lower than that of the Rag-/- recipients (Figure 5B). This was not due to an inadequate amount of OVA antigen production in the GITR-L-/- Rag-/- recipients (Figure 5C). Taken together, the data indicate that GITR-L is required for optimal induction and/or expansion of antigen-specific Treg in the context of hepatic AAV8 gene transfer.

DEPLETION OF CX3CR1+ (GFP) CELLS BY αCD3 IN GITR-L-/- MICE CORRELATES WITH A REDUCED NUMBER OF FoxP3+ TREG CELLS

In vitro expansion of FoxP3+ Treg cells can be achieved by stimulation with a combination of αCD3 and soluble GITR-L (Fc-GITR-L) (9). We then assessed whether injection of αCD3 into WT and GITR-L-/- mice would affect the Treg population. As shown in Figures 6A, B, αCD3 induced a significant reduction of the percentage of FoxP3+ Treg in the spleen and liver of GITR-L-/-CX3CR1(GFP) mice, but not in WT CX3CR1(GFP) mice. In support of our observations in this paper, the reduced number of Tregs coincided with a reduction of CX3CR1+ DCs in the spleen.
and liver of GITR-L−/−CX3CR1(GFP) mice (Figures 6C,D). In contrast, the numbers of CX3CR1+ cells in the spleen and liver were comparable in the two mouse strains under homeostasis (Figure S3 in Supplementary Material).

To further investigate the role of GITR-L in the expansion of FoxP3+ Treg, CD4+ T cells were purified from the spleen of FoxP3(GFP) mice and stimulated in vitro with αCD3 with either Fc-GITR-L or IgG. Forty-eight hours after exposure to αCD3, the number of total CD4+ and FoxP3+CD4+ Treg was significantly higher in the presence of Fc-GITR-L than that of IgG (Figures 7A,B). Interestingly, a subset of CD103+ Treg cells, which is induced in epithelium and in sites of inflammation (23, 34) and comprises approximately 20% of all FoxP3+ Treg cells in the spleen, was also expanded by Fc-GITR-L (Figures 7C,D).

We conclude that while the induction or expansion of Treg is impaired in the absence of GITR-L, Fc-GITR-L provides a positive signal to GITR+ Treg.

DISCUSSION
The receptor-ligand pair GITR/GITR-L (TNFRSF18/TNFSF18) appears to be involved in the development of a variety of inflammation-related diseases in murine models (6, 8, 12, 35, 36). It was originally thought that the suppressor function of Treg cells, which constitutively express GITR, would be abrogated by anti-GITR thus breaking immune self-tolerance (2). More recent additional evidence shows that GITR engagement by its natural ligand GITR-L causes an extensive expansion of functionally competent Tregs (9–11), although the relative role of GITR on Treg and Teff cells remains only partly understood. In this study we find that in the absence of GITR-L the expansion of FoxP3+ Treg cells is impaired in an antigen-specific manner, which can be mimicked by in vivo and in vitro activation of CD4+ Treg cells with αCD3. Our results are consistent with the findings of the Chatila group that expansion and contraction of Teff and Treg dynamically control primary immune responses to foreign antigen (25).

Glucocorticoid-induced TNF receptor family-related protein ligand impacts immune regulation in gene replacement therapy at least at three levels. First, the induction/expansion of antigen-specific Treg cells in the liver after AAV-mediated gene therapy is impaired directly by the absence of GITR-L. Second, the expansion of antigen-specific CD8+ T cells is reduced by GITR-L deficiency. However, impaired expansion of Treg cells can on the other hand up-regulate CD8+ T cell expansion indirectly. Third, GITR-L deficiency affects the infiltration of monocyte-derived MØ to the sites where exogenous protein is expressed and/or the sites of inflammation (30), which changes the local function of different immune cells. These GITR-L-expressing, monocyte-derived MØ may provide a microenvironment for the expression of CD103 in Treg cells, an integrin that facilitates the retention of Treg cells in the sites of inflammation or infection.

Surprisingly, we found that administering αCD3 causes the depletion of CX3CR1+ DCs in the spleen and liver of GITR-L−/−
mice, which correlates with a reduced number of FoxP3+ Tregs. It is reported that IL10-secreting GITR+ Tr1 cells may suppress immune responses by granzyme B-mediated killing of myeloid APCs (37, 38). Granzyme B is also important for the ability of in vitro hi (41). The mechanism how this Ly6C+ an increased expansion of Treg may inhibit this self-destructive suppressive CD8+CX3CR1 the reduction of Treg number during immune responses.

GITR-L-expressing pDCs and MØ (12, 30), may feedback to cause possible that Tr1, Treg, and CD8+ Treg, NK cells, and CD8+ monocytes give rise to CX3CR1+ T cells (40). CX3CR1+ T cells (40). CX3CR1+ T cells are necessary, but not sufficient, for induction of organ-specific autoimmune disease. Expression of GITR-L on hepatic APCs may requires co-stimulation by GITR-L, which enhances Treg induction and function. Expression of GITR-L on hepatic APCs may in part explain the tolerogenic/Treg inducing capacity of hepatic gene transfer.

AUTHOR CONTRIBUTIONS
Gongxian Liao performed all the experiments; Michael S. O’Keeffe helped in processing the samples and editing the manuscript; Guoxing Wang and Boaz van Driel helped in processing the samples and discussing the results. Rene de Waal Malefyt generated GITR-L deficient mice; Hans-Christian Reinecker brought deeper insight into oCD3-inducing murine model. Roland W. Herzog helped in discussing and writing the manuscript; Cox Terhorst is the major organizer of this work and designed the experiments with Gongxian Liao.

ACKNOWLEDGMENTS
We thank Dr. Talal Chatila for providing the FoxP3EGFP knock-in reporter mice, all other members of the Terhorst Lab for helpful discussions. We thank Dr. Shangzhen Zhou and the AAV research vector core at The Children’s Hospital of Philadelphia for the help with production of AA V8-OVA vector. Grant Support: this work was sponsored by National Institutes of Health (P01 HL078810 to Roland W. Herzog and Cox Terhorst, and R01 DK-52510 and P30 DK-43351 to Cox Terhorst).

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at http://www.frontiersin.org/Journal/10.3389/fimmu.2014.00035/abstract

Figure S3 | CX3CR1(GFP)+ phagocytes in spleen and liver leukocytes of CX3CR1(GFP) and GITR-L−/−CX3CR1(GFP) mice under resting condition.

REFERENCES

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 13 November 2013; accepted: 21 January 2014; published online: 03 February 2014.


This article was submitted to Microbial Immunology, a section of the journal Frontiers in Immunology.

Copyright © 2014 Liao, O’Keeffe, Wang, van Driel, de Waal Malefyt, Reinecker, Herzog and Terhorst. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.