signals that profoundly affect DC functions towards $CD4^+$ $CD25^+$ Treg cells. Because NK cell functions are regulated by the balance between inhibitory and activating signals, any future clarification of the role of other NK inhibitory and activating receptors in DC modulation and Treg cell activation will be of great interest.

The cross-presentation of self-antigens by major histocompatibility complex (MHC) class II pathways constitutes an important step towards generating and/or expanding peripheral Treg cells.[30] However, we initially settled our experimental design by using DCs and Treg cells from different donors, and DCs encountered $CD4^+$ T cells in an 'antigen-free' condition. Therefore, Treg cells induced by NK/NH-primed DCs are generated independently of MHC class II-mediated self-antigen recognition. These results give rise to the possibility that the cross-talk of NK cells, DCs and hepatocytes represents an alternative pathway in the generation and expansion of peripheral Treg cells. However, it should be noted that these results may not apply to all donors because of the complexity of the allogeneic system and the relatively few donors tested.

PD-1-mediated suppressive activities were characteristic for $CD4^+$ $CD25^+$ Treg cells generated by NH/IL-2 NK-primed DCs. By contrast, natural $CD4^+$ $CD25^+$ Treg cells exerted their suppressive function, at least in part, in a CTLA-4-dependent fashion. Recent reports have clarified the existence of two subtypes of Treg cells: natural and inducible $CD4^+$ $CD25^+$ Treg cells. Inducible Treg cells exert suppressive activities by using molecular mechanisms distinct from those of natural regulatory cells.[31] Our findings further identify the novel pathways by which inducible $CD4^+$ $CD25^+$ Treg cell activities triggered by NKG2A inhibitory signals are dependent on PD-1-mediated negative costimulation. A recent report identified the interaction of B7 on effector T cells with costimulatory molecules CD28/CTLA-4 on $CD4^+$ $CD25^+$ Treg cells as molecular mechanisms of their suppressor activity.[32] Thus, it is possible that reverse signalling of PDL-1 on effector cells may also be crucial for the negative costimulator-mediated suppressive action of $CD4^+$ $CD25^+$ Treg cells. In the present study, we did not address the mechanisms by which NH/IL-2 NK-primed DCs induce $CD4^+$ $CD25^+$ Treg cells with PD-1-dependent suppressive functions. Further study will be needed to clarify this issue.

We previously showed that NKG2A is expressed at higher levels from NK cells isolated from peripheral blood in patients with chronic hepatitis C virus (HCV) infection than from those in healthy donors.[20] HCV frequently persists in humans, at least in part, due to inefficient induction of NK activity as well as specific T cell responses.[33–35] The small percentage of patients who spontaneously clear the virus and recover from chronic hepatitis C mount vigorous HCV-specific $CD4^+$ and $CD8^+$ T cell responses.[36,37] Research has described an increased frequency of $CD4^+$ $CD25^+$ T cells in the blood of patients with persistent HCV infection compared with those who have spontaneously cleared HCV.[38,39] Our current findings raise the interesting possibility that increased NKG2A expression on NK cells may lead to DC-mediated induction of Treg cells, leading to the inhibition of adaptive responses to HCV and failure to eliminate this virus. Indeed, $CD4^+$ $CD25^+$ T cells induced by HCV-NK/Hep3B hepatoma cell-primed DCs expressed and suppressed effector T cell functions at greater levels than those induced by N-NK/Hep3B-primed DCs (our unpublished data). Interestingly, a recent study identified PD-1-mediated signals as a critical pathway to induce anergic $CD8^+$ T cells and impair antiviral CTL responses in chronic viral infection.[40] In this regard, the therapeutic modification of the PD-1 pathway may synergistically augment antiviral immunity by suppressing Treg activity and recovering CTL responses. It is important to establish whether the PD-1 pathway in liver lymphocytes may be operable *in vivo* and play a critical role in suppression of virus-specific immunity in HCV infection.

In conclusion, we have demonstrated that interaction of NK cells and hepatic cells via NKG2A leads to DC induction of $CD4^+$ $CD25^+$ T cells with PD-1-dependent regulatory activities. These findings also imply that NK receptor signals of NK cells may dictate DC-mediated adaptive immune responses towards tolerogenic or immunogenic status via induction of Treg cells.

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References

1 Shevach EM. $CD4^+$ $CD25^+$ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002; 2:389–400.

2 Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification of functional characterization of human $CD4^+$ $CD25^+$ T cells with regulatory properties isolated from peripheral blood. *J Exp Med* 2001; **193**:1285–94.

3 Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G. *Ex vivo* isolation and characterization of $CD4^+$ $CD25^+$ T cells with regulatory properties from human blood. *J Exp Med* 2001; **193**:1303–10.

4 Belkald Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. $CD4^+$ $CD25^+$ regulatory T cells control Leishmania major persistence and immunity. *Nature* 2002; **420**:502–7.

5 Wang HY, Lee DA, Peng G, Guo Z, Li Y, Kiniwa Y, Shevach EM, Wang RF. Tumour-specific human $CD4^+$ regulatory T cells

and their ligands: implication for immunotherapy. *Immunity* 2004; **20**:107–18.

6 Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD4^+$ $CD25^+$ regulatory cells that control intestinal inflammation. *J Exp Med* 2002; **192**:295–302.

7 Herman AE, Freeman GJ, Mathis D, Benoist C. $CD4^+$ $CD25^+$ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. *J Exp Med* 2004; **199**:1479–89.

8 Khoury SJ, Sayegh MH. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity* 2004; **20**:529–38.

9 Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A. Homeostasis and anergy of CD4 $(^+)$ CD25 $(^+)$ suppressor T cells *in vivo*. *Nat Immunol* 2004; **3**:33–41.

10 Steinman RM, Hawiger D, Nussenzweig D. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; **21**:685–711.

11 Mahnke K, Quan Y, Knop J, Enk AH. Induction of $CD4^+$ $CD25^+$ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood* 2003; **101**:4862–9.

12 Annacker O, Pimenta-Araujo R, Burlen-Defranoux O, Barbosa TC, Cumano A, Bandeira A. $CD4^+$ $CD25^+$ T cells regulate the expansion of peripheral $CD4^+$ T cells through the production of IL-10. *J Immunol* 2001; **166**:3008–18.

13 Yamagiwa S, Gray JD, Hashimoto H, Horwitz DA. A role of TGF-β in the generation and expansion of $CD4^+$ $CD25^+$ regulatory T cells from human peripheral blood. *J Immunol* 2001; **166**:7282–9.

14 Peng Y, Laouar Y, Li MO, Green EA, Flavell RA. TGF-β regulates *in vivo* expansion of Foxp3-expressing $CD4^+$ $CD25^+$ regulatory T cells responsible for protection against diabetes. *Proc Natl Acad Sci USA* 2004; **101**:4572–7.

15 Moretta A. The dialogue between human natural killer cells and dendritic cells. *Curr Opin Immunol* 2005; **17**:306–11.

16 Walzer T, Dalod M, Robbins SH, Zitvogel L, Vivier E. Natural killer cells and dendritic cells: 'L'union fait la force'. *Blood* 2005; **106**:2252–8.

17 Mocikat R, Braumuller H, Gumy A *et al.* Natural killer cells activated by MHC^{LOW} targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity* 2003; **19**:561–9.

18 Van den Broeke LT, Daschbach E, Thomas EK, Andringa G, Berzofsky JA. Dendritic cell-induced activation of adaptive and innate antitumour immunity. *J Immunol* 2003; **171**:5842–52.

19 Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. *Nat Rev Immunol* 2001; **1**:41–9.

20 Jinushi M, Takehara T, Tatsumi T *et al.* Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to the altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. *J Immunol* 2004; **173**:6072–81.

21 Lee N, Goodlett DR, Ishitani A, Marquardt H, Geraghty DE. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. *J Immunol* 1998; **160**:4951–60.

22 Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 1999; **285**:727–9.

23 Pende D, Sivori S, Accame L *et al.* HLA-G recognition by human natural killer cells. Involvement of CD94 both as inhibitory and as activating receptor complex. *Eur J Immunol* 1997; **27**:1875–80.

24 Valerie V, Vosters O, Beuneu C, Nicaise C, Stordeur P, Goldman M. Induction of FOXP3-expressing regulatory $CD4^+$ T cells by human mature autologous dendritic cells. *Eur J Immunol* 2003; **34**:762–72.

25 Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of $CD4^+$ $CD25^+$ regulatory T cells through GITR break immunological self-tolerance. *Nat Immunol* 2002; **3**:135–42.

26 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**:1057–61.

27 Piccioli D, Sbrana S, Melandri E, Valiante NM. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J Exp Med* 2002; **195**:335–41.

28 Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNF-α therapy. *J Exp Med* 2004; **200**:277–85.

29 Wu AJ, Hua H, Munson SH, McDevitt HO. Tumour necrosis factor-α regulation of $CD4^+$ $CD25^+$ T cell levels in NOD mice. *Proc Natl Acad Sci USA* 2002; **99**:12287–92.

30 Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat Immunol* 2005; **6**:1219–27.

31 Bluestone JA, Abbas AK. Natural and adaptive regulatory T cells. *Nat Rev Immunol* 2003; **3**:253–7.

32 Paust S, Lu L, McCarty N, Cantor H. Engagement of B7 on effector T cells by regulatory T cells prevents autoimmune disease. *Proc Natl Acad Sci USA* 2004; **101**:10398–403.

33 Ahmad A, Alvarez F. Role of NK and NKT cells in the immunopathogenesis of HCV-induced hepatitis. *J Leukoc Biol* 2004; **76**:743–59.

34 Golden-Mason L, Rosen HR. Natural killer cells: primary target for hepatitis C virus immune evasion strategies. *Liver Transplant* 2006; **12**:363–72.

35 Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5**:215–29.

36 Lauer GM, Barnes E, Lucas M *et al.* High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* 2004; **127**:924–36.

37 Cox AL, Mosbruger T, Lauer GM, Pardoll D, Thomas DL, Ray S. Comprehensive analysis of $CD8^+$ T cell responses during longitudinal study of acute human hepatitis C. *Hepatology* 2005; **42**:104–12.

38 Cabrera R, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, Nelson DR. An immunomodulatory role for $CD4^+$ $CD25^+$ regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004; **40**:1062–71.

39 Rushbrook SM, Ward SM, Unitt E, Vowler M, Lucas M, Kleneman P, Alexander GJ. Regulatory T cells suppress *in vitro* proliferation of virus-specific $CD8^+$ T cells during persistent hepatitis C virus infection. *J Virol* 2005; **79**:7852–9.

40 Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006; **439**:682–7.

