



**A Critical Role for IL-21 in Regulating Immunoglobulin Production**  
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point, we measured thresholds with only one cue present (monocular texture) and with both cues present (texture and disparity) in sequential blocks of trials. For the cues-inconsistent quadrants, thresholds were lower in the monocular condition than when both disparity and texture were available. The opposite was true in the cues-consistent quadrants. This result illustrates both the benefits (better discrimination when the cues specify changes in the same direction) and the costs (the loss of single-cue information associated with cue combination).

Our data provide a clear demonstration of depth-cue fusion: shape information from texture and disparity cues is combined to form a single, fused percept such that some discriminations that could be made from single-cue estimates are not made (19). We also have evidence for a single, fused percept for shape information from haptics and vision, but in this intermodal case information from single-cue estimates is not lost.

References and Notes

1. Our interest is in the manner in which the nervous system resolves discrepancies at any given moment between sensory measurements. We assume the sensory systems under examination are well calibrated, so their signals will, on average, agree with one another. They will, however, disagree from one measurement to the next due to random measurement error.
2. J. J. Clark, A. L. Yuille, *Data Fusion for Sensory Information Systems* (Kluwer Academic, Boston, MA, 1990).
3. M. S. Landy, L. T. Maloney, E. B. Johnston, M. J. Young, *Vision Res.* **35**, 389 (1995).
4. Z. Ghahramani, D. M. Wolpert, M. I. Jordan, in *Self-Organization, Computational Maps, and Motor Control*, P. G. Morasso, V. Sanguineti, Eds. (Elsevier, Amsterdam, 1997) pp. 117–147.
5. M. O. Ernst, M. S. Banks, *Nature* **415**, 429 (2002).
6. M. S. Landy, H. Kojima, *J. Opt. Soc. Am.* **18**, 2307 (2001).
7. Metamers are composite stimuli that cannot be discriminated even though their constituents can be. The classic example is the inability to discriminate a yellow light consisting of one wavelength from another yellow light consisting of red added to green.
8. W. Richards, *Sens. Processes* **3**, 207 (1979).
9. B. T. Backus, in *Advances in Neural Information Processing Systems*, vol. 14, T. G. Dietterich, S. Becker, Z. Ghahramani, Eds. (MIT, Cambridge, MA, 2002) part VII, chap. 1.
10. E. B. Johnston, B. G. Cumming, M. S. Landy, *Vision Res.* **34**, 2259 (1994).
11. B. T. Backus, M. S. Banks, R. van Ee, J. A. Crowell, *Vision Res.* **39**, 1143 (1999).
12. E. Brenner, W. J. M. van Damme, *Vision Res.* **39**, 975 (1999).
13. With two independent estimators, there are two chances for discriminating the odd stimulus. For each value of the comparison, each estimator has a likelihood of discriminating the comparison from the standard. Complete predictions for independent estimators would therefore include probability summation:  $P(\text{estimator 1 discriminates odd}) + P(\text{estimator 2 discriminates odd}) - P(\text{both discriminate odd})$ . Predicted thresholds that include probability summation would be a rectangle like the one in Fig. 1B, but with rounded corners. Inclusion of probability summation does not affect our interpretation of the data, because probability summation would not produce the observed asymmetries between thresholds in the four quadrants (Figs. 2 and 3).
14. From among the large set of possible tasks and cues, we have examined a small subset. We chose tasks that were natural for participants to perform and pairs of cues that were approximately equally reliable to better show the gains and losses predicted by MLE.

15. Material and methods are available as supporting online material on Science Online.
16. The participants' phenomenology was instructive. They reported using a difference in perceived size when the comparison stimulus was in the cues-consistent quadrants (1 and 3). This percept is well modeled by the equations for combined estimation (Eqs. 1 and 2). Participants' reports were less consistent with stimuli in the cues-inconsistent quadrants (2 and 4). Sometimes they used a difference in perceived size, but frequently they noticed the conflict between the visually and haptically specified sizes and used the perceived conflict to make the oddity discrimination. The phenomenology is consistent with the hypothesis that participants used three estimators in performing the oddity discrimination: two single-cue estimators and a combined estimator.
17. D. C. Knill, *Vision Res.* **38**, 1683 (1998).
18. M. de Berg, M. van Kreveld, M. Overmars, O. Schwarzkopf, *Computational Geometry: Algorithms and Applications* (Springer-Verlag, New York, ed. 2, 2000).
19. The participants' phenomenology was informative. In some trials, they perceived a difference in slant (quadrants 1 and 3). This percept is well modeled by the equations for combined estimation (Eqs. 1 and 2). In other trials, they perceived a difference in the shape of the surface texture. This occurred in the cues-inconsistent quadrants (particularly in the direction for which  $S_c$  is constant, Eq. 3). For example, in the lower right panel of Fig. 2B, the standard's slant was  $-30$  (i.e., left side, far), so the retinal images had smaller and more foreshortened texture on the left. For the four comparison stimuli in quadrant 2, the disparity- and texture-specified slants differed in opposite directions from the standard stimulus.  $\hat{S}_c$

was approximately  $-30$  for these comparison stimuli, but the surface texture was rendered such that the retinal images were nearly equally large (and equally foreshortened) on the left and right. To be consistent with the perceived left-far slant, the surface texture would have to be nonhomogeneous (larger cells on the left and smaller on the right). The odd stimulus was detected by perceiving this nonhomogeneity. Participants were, in this case, using shape-constancy mechanisms (which allow one to determine the shape of markings on a slanted surface). We believe, therefore, that participants did not have access to more than one percept (e.g.,  $\hat{S}_c$ ,  $\hat{S}_t$ , and  $\hat{S}_d$ ); the slant cues were truly fused. They made the discrimination in the cues-inconsistent quadrants by use of another calculation—shape constancy—that allowed them to perceive the objective shape of the texture on the surface. If this hypothesis is correct, metamers (cases in which discrimination is poorer than predicted by single-cue estimates) occurred when the stimulus made the shape-constancy judgment difficult.

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Supporting Online Material

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 Materials and Methods  
 SOM Text  
 Figs. S1 to S4

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## A Critical Role for IL-21 in Regulating Immunoglobulin Production

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The cytokine interleukin-21 (IL-21) is closely related to IL-2 and IL-15, and their receptors all share the common cytokine receptor  $\gamma$  chain,  $\gamma_c$ , which is mutated in humans with X-linked severe combined immunodeficiency disease (XSCID). We demonstrate that, although mice deficient in the receptor for IL-21 (IL-21R) have normal lymphoid development, after immunization, these animals have higher production of the immunoglobulin IgE, but lower IgG1, than wild-type animals. Mice lacking both IL-4 and IL-21R exhibited a significantly more pronounced phenotype, with dysgammaglobulinemia, characterized primarily by a severely impaired IgG response. Thus, IL-21 has a significant influence on the regulation of B cell function in vivo and cooperates with IL-4. This suggests that these  $\gamma_c$ -dependent cytokines may be those whose inactivation is primarily responsible for the B cell defect in humans with XSCID.

The receptor for the lymphoid-specific cytokine IL-21 is expressed on T, B, and NK cells (1, 2). IL-21 was initially reported to have a costimulatory T cell proliferative effect, to augment NK cell expansion and differentiation, and to augment B cell proliferation in response to CD40-specific antibodies, but to inhibit proliferation of B cells stimulated with the combination of IL-4 and IgM-specific antibodies (2). Subsequently, IL-21 was reported not to be required for NK cell development or expansion from murine spleno-

cytes and to oppose certain actions of IL-15 on activated NK cells (3). The receptor for IL-21 contains IL-21R (1, 2) and also shares the common cytokine receptor  $\gamma$  chain ( $\gamma_c$ ) with IL-2, IL-4, IL-7, IL-9, and IL-15 (4, 5). Mutations in  $\gamma_c$  result in XSCID (4, 6), a disease characterized by an absence of T and NK cells and nonfunctional B cells (7). Although the absence of T and NK cells can be explained by defective responses to IL-7 (8–10) and IL-15 (11–13), no cytokine has been linked to the B cell defect. To determine

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whether defective signaling by IL-21 might contribute to this defect, we generated IL-21R<sup>-/-</sup> mice by using a targeting construct that eliminates the IL-21R extracellular and transmembrane domains (fig. S1). IL-21R<sup>-/-</sup> and wild-type mice are similar in the number and phenotype of thymocytes, splenocytes, peritoneal cells, and bone marrow cells, as detected using a panel of antibodies to multiple surface markers (3, 14). IL-21R<sup>-/-</sup> and wild-type splenocytes exhibited similar T cell proliferation in response to CD3-specific antibodies. Similarly, they showed nearly equal B cell proliferation in response to LPS, to

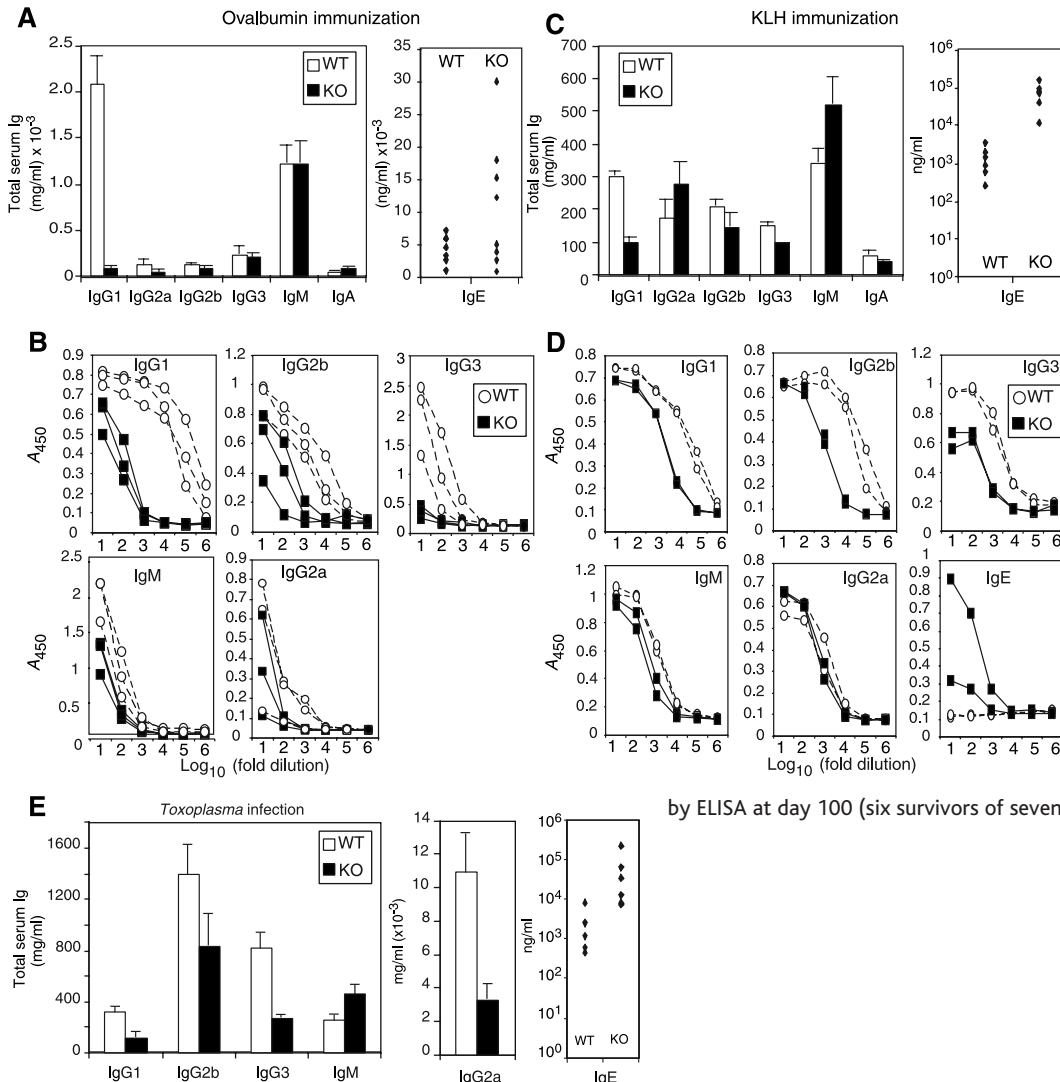
CD40-specific antibodies, and to the combination of IL-4 and IgM-specific antibodies (14). Serum levels of IgG2a, IgG3, and IgM from naïve mice were normal, but the amounts of IgG1 and IgG2b were lower and of IgE were higher in IL-21R<sup>-/-</sup> mice (14). After immunization with ovalbumin, the normal increase in IgG1 seen in wild-type mice was markedly impaired in IL-21R<sup>-/-</sup> mice; total serum IgG1 was 4 to 5% of wild type (Fig. 1A), and antigen-specific IgG1 antibody was about 0.1% of that in wild-type mice (Fig. 1B). There was little, if any, difference in total serum IgG2a, IgG2b, IgG3, IgM, or IgA (Fig. 1A, left), but ovalbumin-specific IgG2b and IgG3 were significantly lower in IL-21R<sup>-/-</sup> mice (Fig. 1B), and total serum IgE levels were variable although markedly higher in some IL-21R<sup>-/-</sup> mice (Fig. 1A, right). Immunization of IL-21R<sup>-/-</sup> mice with keyhole limpet hemocyanin (KLH) also revealed an impaired IgG1 response (Fig. 1C), and KLH-specific IgG1, IgG2b, and IgG3 levels were reduced to about 1/10th of the values for these subclasses in wild-type mice (Fig. 1D,

top). It was noteworthy that IgE production in IL-21R<sup>-/-</sup> mice was markedly increased (50- to 55-fold) (Fig. 1C), as was KLH-specific IgE (Fig. 1D). Normal mice injected with IgD-specific antibodies respond by day 8 with marked elevations of serum IgG1 and IgE (15). In IL-21R<sup>-/-</sup> mice, IgG subclasses were similar to those in wild-type mice (IgG1 tended to be lower, but the difference was not statistically significant); however, IgE responses of IL-21R<sup>-/-</sup> mice were increased about 10-fold (fig. S2).

Infection with *Toxoplasma gondii* induces a striking T helper cell type 1 (T<sub>H</sub>1) CD4<sup>+</sup> T cell-dependent interferon- $\gamma$  (IFN- $\gamma$ ) response that is required for survival during the acute phase of infection (16). Serum IFN- $\gamma$  levels were similar in wild-type and IL-21R<sup>-/-</sup> mice 5 days after infection, as were IFN- $\gamma$  levels after in vitro culture with *Toxoplasma* antigen (17). Correspondingly, six of seven mice in both wild-type and IL-21R<sup>-/-</sup> groups survived more than 100 days. Despite similar survival, at day 100 in the IL-21R<sup>-/-</sup> mice, serum IgG1 was about one-

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**Fig. 1.** Total and antigen-specific immunoglobulin induced by immunization with ovalbumin (A and B) or KLH (C and D) in wild-type and IL-21R knockout (KO) mice. Shown are the total immunoglobulin for each subclass (A and C) and relative amount of antigen-specific immunoglobulin subclasses (B and D) induced by immunization. In (A) and (B), ovalbumin was injected intraperitoneally twice, and after an additional week, serum immunoglobulin levels were assessed by enzyme-linked immunosorbent assay (ELISA). For (C) and (D), KLH was injected once into foot pads, bottom of tail, and back, and at day 9, serum immunoglobulin levels were determined. In (A) and (C), shown are the means  $\pm$  SEM of eight and six mice, respectively, in each group. (B) and (D) each show results from one of three experiments. (E) Diminished IgG subclasses and increased IgE after infection with *Toxoplasma gondii*. *T. gondii* (ME-49 strain) was intraperitoneally injected at a dose of 20 cysts per mouse, and serum immunoglobulin was determined by ELISA at day 100 (six survivors of seven mice in each group were analyzed).

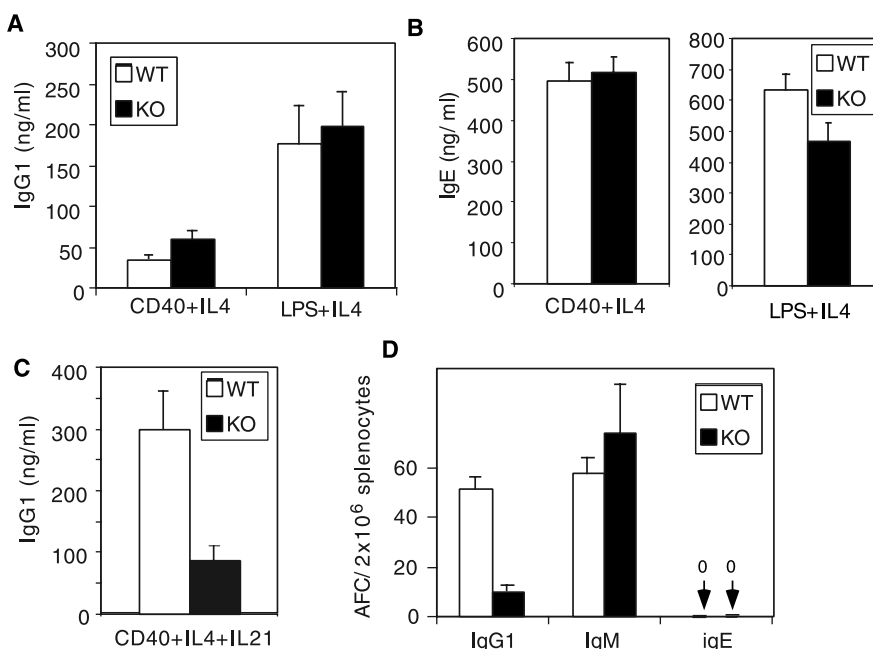
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third that seen in wild-type mice (Fig. 1E, left), whereas IgE was increased about 20- to 25-fold (Fig. 1E, right). This increased IgE is noteworthy given that there is not normally an IgE response in mice to *Toxoplasma gondii* (18). IgG2a, IgG2b, and IgG3 were lower than in wild-type mice (Fig. 1E).

To further evaluate the impaired IgG1 and augmented IgE response, splenic CD4<sup>+</sup> T cells were stimulated with plate-bound CD3-specific antibody under T<sub>H</sub>1- or T<sub>H</sub>2-polarizing conditions, but neither IFN- $\gamma$  nor IL-4 production was diminished (fig. S3A), suggesting that these differentiation pathways are not impaired in these mixed background (129  $\times$  C57BL/6) IL-21R<sup>-/-</sup> mice. Moreover, when mice were immunized with KLH and spleen cells were restimulated *in vitro*, IFN- $\gamma$  (fig. S3B) and IL-4 (fig. S3C) levels, as well as proliferation (fig. S3D), were relatively similar in IL-21R<sup>-/-</sup> and wild-type mice. Spleen cells from IL-21R<sup>-/-</sup> and normal mice generated comparatively similar levels of IFN- $\gamma$  (fig. S3E) and IL-4 (fig. S3F) after 4 days of stimulation on plates coated with 2C11 monoclonal antibody to CD3 $\epsilon$ .

To determine whether the impaired IgG1 response and augmented IgE production resulted from an obvious B cell defect, we cultured purified B cells with either the combination of CD40-specific antibodies plus IL-4 or LPS plus IL-4. As expected, production of IgG1 (Fig. 2A) and IgE (Fig. 2B) was relatively similar in IL-21R<sup>-/-</sup> and wild-type B cells, consistent with the lack of a requirement of IL-21R for stimulation of B cells by these stimuli. However, the combination of CD40-specific antibodies plus IL-4 and IL-21 boosted IgG1 production in purified wild-type B cells to a significantly higher level than in B cells from IL-21R<sup>-/-</sup> mice (Fig. 2C). Thus, IL-21 affects IgG1 production in normal murine B cells, and IL-21R<sup>-/-</sup> mice have an intrinsic B cell defect.

To evaluate the ability of IL-21R<sup>-/-</sup> mice to develop antibody-forming cells and memory B cells, we immunized mice with a T cell-dependent antigen, nitrophenyl (NP)-conjugated KLH. After exposure to this antigen, we evaluated the number of antibody-forming cells as hapten (NP)-specific antibody-secreting cells and memory B cells as NP-specific antibody-expressing cells that did not express IgD, CD4, CD8, or F4/80 and that did not stain with propidium iodide (19, 20). In wild-type and IL-21R<sup>-/-</sup> mice, there was at most only a modest difference in the number of splenic memory cells (fig. S4), but the number of hapten-specific IgG1-forming cells in the spleen was much lower in the IL-21R<sup>-/-</sup> mice (Fig. 2D). Thus, the decreased IgG1 could not be explained simply by a decrease in memory cells and, instead, likely reflects diminished antigen-specific IgG1-producing cells. Serum NP-specific IgG1 was also diminished (14).

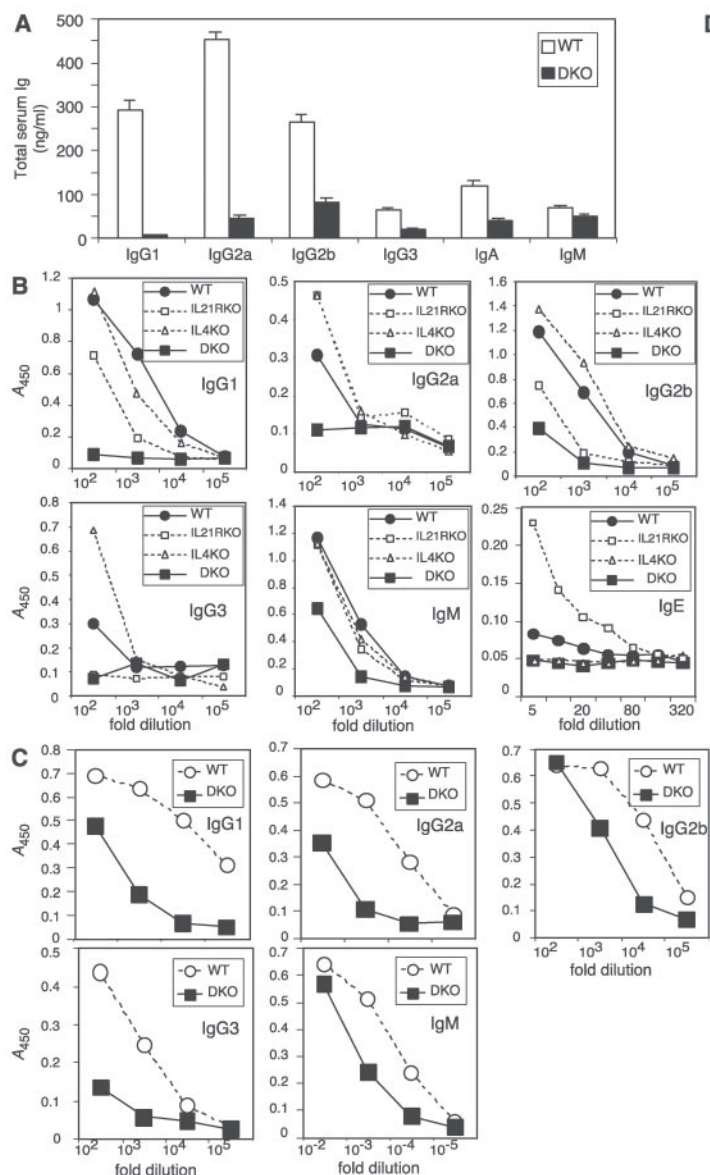


**Fig. 2.** *In vitro* IgG1 and IgE production in IL-21R<sup>-/-</sup> mice. (A and B) B cells were stimulated with IL-4 plus either CD40-specific antibodies or LPS. Production (means  $\pm$  SEM) of IgG1 at day 4 (A) and IgE at day 7 (B) were measured by ELISA. (C) B cells from wild-type and IL-21R<sup>-/-</sup> mice were stimulated with the combination of CD40-specific antibodies + IL-4 + IL-21. IgG1 was measured at day 4. In B cells from wild-type mice, the combination of CD40-specific antibodies + IL-4 + IL-21 also gave higher IgG1 production than did CD40-specific antibodies + IL-4 without the addition of IL-21 (14). (D) NP-KLH was mixed with Ribi adjuvant and injected intraperitoneally. Twelve days later, the number of NP-specific IgG1-, IgM-, and IgE-forming cells in splenocytes was determined by ELISPOT. Shown are means  $\pm$  SEM from four IL-21R<sup>-/-</sup> and three wild-type mice. The levels of IgE-forming cells were below the limits of detection.

Mice lacking  $\gamma_c$  exhibit a marked defect in B cell development (21, 22) as a result of defective IL-7 signaling (8, 9), whereas humans with XSCID have normal numbers of B cells (4), presumably because of IL-7-independent signals that mediate human B cell development (4, 10). Nevertheless, humans with XSCID exhibit markedly defective B cell function (23–26). IL-21R<sup>-/-</sup> murine B cells are functionally abnormal but not as defective as in human XSCID. We hypothesized that if IL-7 signaling were left intact, but if signaling by another  $\gamma_c$ -dependent cytokine in addition to IL-21 were also inactivated, it might be possible to generate a phenotype in mice more closely resembling that found in human XSCID. Given the importance of IL-4 for B cell function, we explored the effect that deficient IL-4 signaling might have in the context of IL-21R deficiency using IL-4<sup>-/-</sup>IL-21R<sup>-/-</sup> double-knockout (DKO) mice. As with IL-21R<sup>-/-</sup> mice, no gross developmental defects were observed compared with wild-type mice (fig. S5, A and B). However, naïve IL-4<sup>-/-</sup>IL-21R<sup>-/-</sup> mice exhibited low serum levels of IgA and IgG subclasses, most strikingly in IgG1 (Fig. 3A). In contrast, serum IgM levels were relatively normal, which is analogous to observations made in humans with XSCID.

To further investigate immunoglobulin

production by these animals, we immunized them with trinitrophenyl-conjugated chicken  $\gamma$ -globulin (TNP-CGG), and the IL-4<sup>-/-</sup>IL-21R<sup>-/-</sup> mice showed severely impaired production of TNP-specific IgG1, IgG2a, IgG2b, and IgG3, and only about 10 to 20% of normal levels of IgM production (Fig. 3B). Strikingly, the strong up-regulation of IgE seen in the IL-21R<sup>-/-</sup> mice completely disappeared in IL-4<sup>-/-</sup>IL-21R<sup>-/-</sup> mice (Fig. 3B, lower right panel), which indicated that the increased IgE in IL-21R<sup>-/-</sup> mice was strictly dependent on IL-4. We further evaluated the ability of the IL-4<sup>-/-</sup>IL-21R<sup>-/-</sup> mice to respond to two other antigens. KLH induced a similar immunoglobulin profile to TNP-CGG (Fig. 3C), showing severely impaired production of KLH-specific IgG1, IgG2a, and IgG3, and only about 10 to 20% of normal levels of IgG2b and IgM production. The response to NP-KLH was also impaired, although the defects in IgG1, IgG2a, and IgG3 responses were less severe (14). Although germinal centers were relatively normal in mice lacking either IL-4 or IL-21R (14), in IL-4<sup>-/-</sup>IL-21R<sup>-/-</sup> mice germinal centers were disorganized, with decreased numbers of apoptotic bodies in macrophages (Fig. 3D), consistent with diminished somatic mutation and defective affinity maturation. These results indicate an important cooperative role for



**Fig. 3.** Defective immunoglobulin production in IL-4<sup>-/-</sup> IL-21R<sup>-/-</sup> mice correlates with disorganized germinal center formation. (A) Defective immunoglobulin levels in IL-4<sup>-/-</sup> IL-21R<sup>-/-</sup> naive mice. (B) Serum TNP-specific immunoglobulin levels were measured by ELISA 10 days after peritoneal injection of TNP-CGG in complete Freund's adjuvant (CFA) in wild-type, IL-21R<sup>-/-</sup>, IL-4<sup>-/-</sup>, and IL-4<sup>-/-</sup> IL-21R<sup>-/-</sup> (DKO) mice. Shown is a representative result of four independent experiments. IL-4<sup>-/-</sup> mice on the C57BL/6 background were from Jackson Laboratory. (C) Serum KLH-specific immunoglobulin levels were measured by ELISA 10 days after a peritoneal injection of KLH in CFA. Shown is one of three independent experiments; overall, one of six IL-4<sup>-/-</sup> IL-21R<sup>-/-</sup> mice did not show a decrease in IgG2a, whereas the other five mice were as shown in the Figure. (D) Defective germinal center formation in IL-4<sup>-/-</sup> IL-21R<sup>-/-</sup> mice. Mice were injected with 100 μg TNP-CGG with CFA. The wild-type (WT) lymph node contains a well-organized germinal center (outlined) with blast cells and tingible body macrophages containing apoptotic bodies (arrows). The IL-4<sup>-/-</sup> IL-21R<sup>-/-</sup> DKO lymph nodes had barely recognizable, poorly organized germinal center-like areas with scattered apoptotic cells (arrowheads). The wild-type and DKO lymph nodes were readily distinguished by a histopathologist in a blinded fashion on the basis of the abnormal germinal centers in the DKO mice.

IL-4 and IL-21 for normal germinal center function.

Although IL-4 plays an essential role for IgE class switching, residual IgG1 production in IL-4<sup>-/-</sup> or Stat6<sup>-/-</sup> mice suggests the existence of additional regulator(s) of IgG1 production. We demonstrate that IL-21 is such a molecule. It is noteworthy that, in IL-21R<sup>-/-</sup> mice, IgE production is elevated, but IgG1 is diminished. This potentially results from favored utilization of a  $\gamma$ 1-independent mechanism of  $\mu$  to  $\epsilon$  immunoglobulin class switch, or alternatively, class switching might proceed so rapidly from  $\mu$  via  $\gamma$ 1 to  $\epsilon$  that IgG1 levels are diminished. Other mechanisms for the increased IgE could also exist.

Humans or dogs with XSCID can mount minimal specific IgM responses but almost no IgG response (27). In some patients lacking evidence of donor B cell engraftment, B cell

function can be normalized after bone marrow transplantation (28). However, it is conceivable that small numbers of donor B cells may in fact have engrafted in this situation, perhaps outside the peripheral blood (e.g., in lymph nodes) and can thus respond to  $\gamma$ c-dependent cytokines, particularly since the detection of B cell chimerism can be difficult. Other data strongly support an intrinsic B cell defect in XSCID. First, in XSCID carrier females, who have normal T cell function, immature surface IgM<sup>+</sup> B cells exhibit random X chromosome inactivation, but more mature, terminally differentiated IgM<sup>-</sup> B cells preferentially exhibit nonrandom X inactivation (29), which suggests that only B cells expressing the wild-type X chromosome become antibody-producing cells. Second, other studies have concluded that effective humoral reconstitution can only be achieved by the engraftment of normal donor B cells (30), so

that if donor B cells do not engraft, patients typically require long-term intravenous  $\gamma$ -globulin (31). In this regard, in data collated in 2002, of 43 SCID patients who received hematopoietic stem cell transplantation at Duke University Medical Center, the 20 who exhibited normal B cell function either had documented donor B cell engraftment (18 patients) or have not been adequately evaluated (2 patients); moreover, effective response to immunization with  $\Phi$ X174 was only found when donor B cells engrafted (31). This is in contrast to the situation in humans with SCID resulting from IL-7R deficiency, in which intrinsic B cell function is readily restored by engrafted T cells (32), and no intrinsic B cell defect has been noted. As noted above, in humans, IL-7 signaling can be inactivated without loss of B cell development, whereas IL-7 is vital for B cell development in mice (4). By generating mice defective in IL-4

and IL-21 signaling but which retain IL-7 signaling and thus B cell development, we have established in mice a phenotype that appears to closely resemble that of B cells from patients with XSCID, suggesting that defective signaling by IL-4 and IL-21 might explain the B cell defect in XSCID.

References and Notes

1. K. Ozaki, K. Kikly, D. Michalovich, P. R. Young, W. J. Leonard, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11439 (2000).
2. J. Parrish-Novak et al., *Nature* **408**, 57 (2000).
3. M. T. Kasaian et al., *Immunity* **16**, 559 (2002).
4. W. J. Leonard, *Nature Rev. Immunol.* **1**, 200 (2001).
5. H. Asao et al., *J. Immunol.* **167**, 1 (2001).
6. M. Noguchi et al., *Cell* **73**, 147 (1993).
7. R. H. Buckley et al., *J. Pediatr.* **130**, 378 (1997).
8. U. von Freeden-Jeffry et al., *J. Exp. Med.* **181**, 1519 (1995).
9. J. J. Peschon et al., *J. Exp. Med.* **180**, 1955 (1994).
10. A. Puel, S. F. Ziegler, R. H. Buckley, W. J. Leonard, *Nature Genet.* **20**, 394 (1998).

11. M. Cavazzana-Calvo et al., *Blood* **88**, 3901 (1996).
12. M. K. Kennedy et al., *J. Exp. Med.* **191**, 771 (2000).
13. J. P. Lodolce et al., *Immunity* **9**, 669 (1998).
14. K. Ozaki et al., unpublished observations.
15. F. D. Finkelman, C. M. Snapper, J. D. Mountz, I. M. Katona, *J. Immunol.* **138**, 2826 (1987).
16. G. S. Yap, A. Sher, *Immunobiology* **201**, 240 (1999).
17. C. G. Feng, unpublished observations.
18. Y. Suzuki et al., *J. Immunol.* **157**, 2564 (1996).
19. L. J. McHeyzer-Williams, M. Cool, M. G. McHeyzer-Williams, *J. Exp. Med.* **191**, 1149 (2000).
20. D. J. Driver, L. J. McHeyzer-Williams, M. Cool, D. B. Stetson, M. G. McHeyzer-Williams, *J. Immunol.* **167**, 1393 (2001).
21. J. P. DiSanto, W. Muller, D. Guy-Grand, A. Fischer, K. Rajewsky, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 377 (1995).
22. X. Cao et al., *Immunity* **2**, 223 (1995).
23. T. N. Small et al., *Hum. Immunol.* **25**, 181 (1989).
24. M. L. Gougeon et al., *J. Immunol.* **145**, 2873 (1990).
25. M. E. Conley, *Clin. Immunol. Immunopathol.* **61**, S94 (1991).
26. R. H. Buckley et al., *FASEB J.* **7**, 95A (1993).
27. B. J. Hartnett et al., *Vet. Immunol. Immunopathol.* **75**, 121 (2000).

28. E. Haddad et al., *Blood* **94**, 2923 (1999).
29. M. E. Conley et al., *Proc. Natl. Acad. Sci. U.S.A.* **85**, 3090 (1988).
30. H. White, A. Thrasher, P. Veys, C. Kinnon, H. B. Gaspar, *Eur. J. Immunol.* **30**, 732 (2000).
31. R. H. Buckley, personal communication.
32. R. H. Buckley et al., *N. Engl. J. Med.* **340**, 508 (1999).
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Supporting Online Material

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Materials and Methods

Figs. S1 and S2

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# The Domestication of Social Cognition in Dogs

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Dogs are more skillful than great apes at a number of tasks in which they must read human communicative signals indicating the location of hidden food. In this study, we found that wolves who were raised by humans do not show these same skills, whereas domestic dog puppies only a few weeks old, even those that have had little human contact, do show these skills. These findings suggest that during the process of domestication, dogs have been selected for a set of social-cognitive abilities that enable them to communicate with humans in unique ways.

Recent research has shown that primates possess a number of sophisticated social-cognitive skills, with some theories of cognitive evolution predicting that highly social primates are special in this regard (1, 2). For example, many species of nonhuman primate follow the gaze direction of conspecifics and humans to outside objects—an adaptive social-cognitive skill for vicariously detecting food, predators, and important social interactions among group mates (3). Chimpanzees even follow the gaze direction of humans past distracting stimuli and behind barriers to a specific target, and they also understand that another individual cannot see something if its perspective is occluded by a barrier, thus demonstrating a fairly sophisticated understanding of how the visual perception of others works (4–6).

Curiously, however, there is one task involving gaze-following at which chimpanzees and other primates perform poorly. In the so-called object choice task, an experimenter hides a piece of food in one of two opaque containers, and the subject, who did not see where the food was hidden, is allowed to choose only one. Before presenting the subject with the choice, the experimenter gives a communicative cue indicating the food's location, for example, by looking at, pointing to, tapping on, or placing a marker on the correct container. The majority of primates, as individuals, do not spontaneously perform above chance levels on this task, no matter what the cue [although for possible exceptions, see (7, 8)], and those who eventually perform well typically take dozens of trials or more to learn (9–17). In addition, when primates have been tested in more difficult tests that require them to show flexible use of social cues (such as with novel or arbitrary social cues), without exception they do not use the cues provided (10, 11, 15).

In contrast, the majority of domestic dogs (*Canis familiaris*) tested in the object choice paradigm effectively use many different vi-

sual cues presented by humans (such as looking at, pointing to, or touching the correct container). Dogs have even shown the ability to use novel social cues to find hidden food; for example, a human placing a physical marker on the correct container. They also are successful in more difficult tests, when a human moves toward the incorrect choice while giving the cue or when the cues are presented statically (for example, the dog enters the room to see a human or conspecific already looking at or pointing at the correct food location). Many dogs are skillful from the first trial, with no learning effects being observed within the experiment. Controls have ruled out the possibility that dogs use olfactory cues to find the hidden food (18–22). Although it seems from these studies that dogs are more skillful than primates in using human social cues to find hidden food, there has yet to be a direct comparison between the ability of dogs and that of any primate species in their use of human social cues. Thus, in the first experiment, we compared chimpanzees (*Pan troglodytes*) and dogs (*C. familiaris*) in an object choice task using a common methodology.

Another obvious question is how domestic dogs have acquired their skill in using human social cues. One hypothesis is that canids in general are unusually flexible in the types of social information they are capable of exploiting. For example, wolves, the closest relative of dogs (23), typically live in cooperatively hunting social groups, making it likely that they need to exploit the behavior of conspecifics and quarry alike, and this ability may then generalize to humans (19). The canid generalization hypothesis predicts that many canids (especially wolves) should perform at least as well as dogs on social tasks, as has been found previously with non-social tasks (24, 25). Another hypothesis is that domestic dogs have much more experi-

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