Human B lymphocytes can produce nine different isotypes of antibody including IgM, IgD, IgG1-4, IgA1-2, and IgE. Each of the four subclasses of human IgG molecules, numbered according to their relative frequency in serum, exhibit a unique profile of effector functions allowing the elimination of pathogenic agents through different mechanisms (1). The IgG1 and IgG3 are predominantly produced in response to protein antigens (2) but chronic stimulation with such antigens results in an increased proportion of IgG4 (1). In contrast, carbohydrate antigens most often induce IgG2 responses (2). A certain commonality in cytokine regulation of isotype switching between human and murine can be noted. In both species, IL-4 directs switching to IgE secretion (3, 4) and mice with a disrupted IL-4 gene are unable to produce IgE (5). TGF-β is involved in switching towards IgA in both species, although addition of IL-10 is necessary for human B cells (6, 7). IFN-γ is able to induce LPS-activated B cells to secrete IgG2a (8) and dextran-conjugated anti-IgD activated B cells to produce both IgG2a and IgG3 (9). In vivo studies show that administration of IFN-γ stimulates the IgG2a response. Likewise, mice treated with neutralizing anti-IFN-γ antibodies (10) and mice whose IFN-γ receptor gene has been inactivated (11), show a strong reduction of IgG2a responses. Evolution by duplication of a common ancestral γ chain gene in both species yielded four different IgG subclasses, but this occurred earlier for the mouse and later in evolution for humans. Thus, different mechanisms are likely to be used for IgG subclass regulation in mice and in humans. However, it is interesting that IL-4 induces switching towards mouse IgG1 (12) and human IgG4 (13) which are both noncomplement-fixing subclasses. Apart from IL-4 (13) and IL-13 (14) which are involved in the switch towards IgG4, the cytokines that direct the switch towards human IgG subclasses have not been identified. Activation through CD40 allows proliferation but not antibody secretion of naive surface (s)IgD + IgM + B cells and further addition of particles of Staphylococcus aureus (SAC) enhances their growth and induce them to secrete high levels of IgM. In the presence of IL-10, naive sIgD + sIgM + B cells activated through CD40 are induced to secrete large amounts of IgM, IgG1, and IgG3, when activated through CD40 in the presence of IL-10. Thus, in addition to its growth-promoting and differentiating activities on human B cells, IL-10 may represent a switch factor for IgG1 and IgG3.
Table 1. Unseparated B Cells Cultured in the CD40 System Secrete IgG1, IgG2, and IgG3 in Response to IL-10

| IL-10 | IgG1 | IgG2 | IgG3 | IgG4 | IgM |
|-------|------|------|------|------|-----|
| -     | <50  | <90  | <65  | <30  | 50  |
| +     | 68,520 ± 1,050 | 14,235 ± 500 | 1,830 ± 280 | <30 | 20,500 ± 2,300 |

Purified tonsillar B cells (5 x 10⁴ cells/well; >99% CD20⁺) were cultured in flat-bottom microwells for 10 d on 5 x 10³ irradiated CDw32 L cells with 0.5 µg/ml mAb89 without or with 100 ng/ml IL-10. Supernatants were harvested after 10 d and Ig levels were determined by ELISA. Results are means ± SD of quadruplicates culture. Representative of one out of three experiments.
Figure 1. IL-10 induces anti-CD40 activated naive slgD⁺ slgM⁺ B cells to secrete high levels of IgM and IgG. (A and B) $5 \times 10^4$ MACS⁺-purified slgD⁺ B cells were cultured for 10 d with $5 \times 10^3$ irradiated CDw32 L cells with mAb89 without or with 100 ng/ml IL-10. The results of eight experiments performed over a 2-yr period are shown. (C) B cells were cultured with medium alone, 10 ng/ml IL-1α, 100 ng/ml IL-1β, 20 U/ml IL-2, 10 ng/ml IL-3, 50 U/ml IL-4, 10 ng/ml IL-5, 200 ng/ml IL-6, 10 ng/ml IL-7, 100 ng/ml IL-10, 0.5 ng/ml TGF-β1, 500 U/ml IFN-γ, and 2.5 ng/ml TNF-α. Supernatants were harvested after 10 d and IgM and IgG levels were determined by ELISA. Results are means of quadruplicate cultures. 

A more detailed analysis of IgG production demonstrated a differential effect of IL-10 on the various IgG subclasses. IL-10 induced naive slgD⁺ B cells to synthesize exclusively IgG1 and IgG3 (Fig. 2). After 3 wk of culture, IgG1 and IgG3 production by slgD⁺ B cells reached a maximum (5,750 and 4,400 ng/ml, respectively) whereas these cells secreted <90 ng/ml IgG2 and <50 ng/ml IgG4. The lack of IgG2 production was not due to inhibition by IL-10 since IL-10 induced high levels of IgG2 from the isotype-committed slgD⁻ B cell population (Fig. 2). The difference between IgG2 levels induced in naive and isotype-committed populations also indicates that contamination of the naive B cell population by isotype-committed B cells was minimal. Thus, the high levels of IgG1 and IgG3 produced by the naive B cells in response to IL-10 strongly suggest that they result from isotype switch. The kinetics of IgG1 and IgG3 production were also consistent with switching, since isotype-committed B cells secreted 5,300 ng/ml IgG1 and 540 ng/ml IgG3 as early as 7 d after stimulation whereas these isotypes remained undetectable in the supernatants of naive B cells. The delay in IgG subclasses secretion is consistent with the time required for the genetic recombination within the H chain locus to occur. This IgG1- and IgG3-inducing activity of IL-10 was not related to the organ origin of the B cells as identical results had been obtained with B cells isolated from spleens (data not shown).
Since both IL-10 and anti-CD40 were required for the secretion of IgM as well as IgG1 and IgG3, the results presented above do not allow discrimination between the respective roles of IL-10 and anti-CD40 in inducing the actual isotype switch. To distinguish between these two potential switch signals, we used S. aureus Cowan I strain (SAC) as a costimulus in the anti-CD40 system. Addition of SAC resulted in the induction of considerable IgM secretion (30,000 ng/ml) by naive tonsillar B cells, but IgG levels were still undetectable (Fig. 3 A). When IL-10 was added to the anti-CD40/SAC system, IgM secretion was moderately enhanced (fourfold), whereas IgG1 and IgG3 were strongly induced (from undetectable values to 2,910 and 3,030 ng/ml, respectively), and IgG2 or IgG4 were still undetectable. By comparison, unseparated B cells activated through CD40 and SAC secreted considerable amounts of IgM but also IgG1, IgG2, and IgG3, without adding exogeneous cytokines. Addition of IL-10 to the anti-CD40/SAC system further enhanced the production of IgM (25-fold), IgG1 (threefold), IgG2 (2.6-fold), and IgG3 (40-fold) from unseparated B cells (Fig. 3 B). These results indicate that IL-10 rather than anti-CD40 was responsible for the secretion of IgG1 and IgG3 and that the effect on IgG3 was particularly striking on naive B cells as well as on unseparated B lymphocytes. Furthermore, limiting dilution analysis indicated that IL-10 acted through increasing the frequency of slgD + B cells secreting either IgG1 (1:1,800) or IgG3 (1:600) in the anti-CD40/SAC system, whereas in the absence of IL-10, <1:5,000 B cells produced IgG1 or IgG3 (data not shown).

Cells isolated from cord blood were used as another source of B lymphocytes, since this population does not contain detectable numbers of isotype-committed cells. However, neonatal B cells, which are deficient in their ability to produce...
Igs, can be induced to switch after T cell activation (20). As shown in Fig. 4 A, neonatal B lymphocytes isolated from cord blood behaved similarly to naive cells purified from tonsils. None of the IgG subclasses were produced when cord blood B cells were cultured in anti-CD40/SAC system alone. Addition of IL-10 resulted in the production of considerable amounts of both IgG1 and IgG3 but not IgG2 and IgG4. The Hyper-IgM syndrome was recently demonstrated to be due to a defective CD40 ligand on T cells, accounting for their inability to drive an isotype switch (16, 21, 22). B cells from these patients can be induced to switch isotype in vitro when cocultured with a malignant human T cell line (23) or after CD40 triggering in the presence of cytokine (16, 21). As shown in Fig. 4 B, PBMC from one such patient cultured in the anti-CD40/SAC system behaved similarly to naive B cells from normal individuals. They did not secrete IgG when stimulated with anti-CD40/SAC system alone, but secreted IgG1 and IgG3 but neither IgG2 nor IgG4 after addition of IL-10.

Taken together, the present data support the notion that IL-10 induces naive sIgD+ B cells to switch towards IgG1 and IgG3 because (a) naive B cells exclusively synthesize IgG1 and IgG3 in response to IL-10 and do not produce IgG2; (b) different sources of naive B cells yield the same isotypes; and (c) naive B cells produced these isotypes with a delayed kinetics when compared to isotype-committed B cells.

The CD40 system shares many of the important features occurring within the germinal centers of the lymphoid follicles. Indeed, CD40 activation was sufficient to restore the switching capacity of Hyper-IgM B cells in response to cytokines. Thus, the triggering of CD40 plays a key role in isotype switching, possibly by turning on the isotype switch machinery, which supposedly alters the DNA structure of the H chain locus. Cytokines, on the other hand, are considered to provide isotype specificity. Indeed, IL-10 alone induces switching towards IgG1 and IgG3, and together with TGF-β towards IgA (7), whereas IL-4 and IL-13 induce IgE and IgG4. Yet to be resolved is the cytokine(s) that induces the switch towards IgG2, an isotype most often found in response to carbohydrate antigens (2).

This study brings up a novel activity of IL-10, namely its ability to induce human naive B cells to switch isotype from IgM to IgG1 and IgG3. This function is additional to IL-10's B cell differentiation activity (7, 19) that induces isotype-committed sIgD+ B cells to secrete IgG1, IgG2, and IgG3. Isotype switching was induced in different sources of human naive B cells including highly purified tonsillar or splenic sIgD+ B cells, neonatal B, and B cells from the Hyper-IgM patient. Further studies at the molecular level are required to determine the respective contribution of IL-10, CD40 triggering, and SAC. It is possible that IL-10 may not account for all IgG3 and particularly IgG1 switch-inducing activity. The ratio IgG1/IgG3 is close to 1 in culture supernatants of naive adult B and in neonatal B cells, whereas it is close to 10 in culture supernatants of unseparated B cells and in serum. The in vivo prevalence of IgG1 could be due to the existence of another factor inducing or enhancing switching to IgG1. In this context, several Ig isotypes are under the control of redundant molecules, such as switching to human IgG4 and IgE in response to IL-4 or IL-13. It is interesting to note that the IgG1 and IgG3 isotypes whose switch is directed by IL-10, preferentially bind to those Fcγ receptors (FcyRI, II, and III) whose expression are modulated on monocytes by IL-10 (24). Finally, this study raises the possibility that patients suffering from IgG3 and IgG1 deficiencies may either display reduced IL-10 secretion, produce IL-10 antagonists, or show an intrinsic B cell impairment resulting in defective IL-10 responses.

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References

1. Jefferis, R., and D.S. Kumararatne. 1990. Selective IgG subclass deficiency: quantification and clinical relevance. Clin. Exp. Immunol. 81:357.

2. Hammarström, L., and C.I.E. Smith. 1986. IgG subclasses in bacterial infections. Monogr. Allergy. 19:122.

3. Péne, J., F. Rousset, F. Brière, I. Chrétien, X. Paliard, J. Banchereau, H. Spits, and J.E. De Vries. 1988. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by interleukin 4 and suppressed by interferon γ. J. Immunol. 141:1218.

4. Coffman, R.L., and J. Carty. 1986. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-γ. J. Immunol. 136:949.

5. Köhn, K., K. Rajewsky, and W. Müller. 1991. Generation and analysis of interleukin-4 deficient mice. Science (Wash. DC). 254:707.

6. Coffman, R.L., D.A. Lebman, and B. Shadrer. 1989. Transforming growth factor β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. J. Exp. Med. 170:1039.
7. Defrance, T., B. Vanbervliet, F. Brière, I. Durand, F. Rousset, and J. Banchereau. 1992. Interleukin 10 and transforming growth factor β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. J. Exp. Med. 175:671.

8. Snapper, C.M., and W.E. Paul. 1987. Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science (Wash. DC). 236:944.

9. Snapper, C.M., T.M. McIntyre, J.M. Pecanha, F.D. Finkelman, A. Lees, and J.J. Mond. 1992. Induction of IgG3 secretion by Interferon γ: a model for T cell-independent class switching in response to T cell-independent type 2 antigens. J. Exp. Med. 175:1367.

10. Finkelman, F.D., I.M. Katona, T.R. Mosmann, and R.L. Coffman. 1988. Interferon-γ regulates the isotypes of immunoglobulin secreted during in vivo humoral immune responses. J. Immunol. 140:1022.

11. Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilcek, R.M. Zinkernagel, and M. Aguet. 1993. Immune response in mice that lack the interferon-γ receptor. Science (Wash. DC). 259:1742.

12. Isakson, P.C., E. Pure, S. Vitetta, and P.H. Krammer. 1982. T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. J. Exp. Med. 155:734.

13. Lundgren, M., U. Persson, P. Larsson, C. Magnusson, C.I.E. Smith, L. Hammarström, and E. Severinson. 1989. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. Eur. J. Immunol. 19:1311.

14. Punnonen, J., G.G. Aversa, B.G. Cocks, A.N.J. McKenzie, S. Menon, G. Zurawski, R. de Waal Malefyt, and J.E. de Vries. 1993. Interleukin-13 induces interleukin-4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc. Natl. Acad. Sci. USA. 90:3750.

15. Rousset, F., E. Garcia, and J. Banchereau. 1991. Cytokine-induced proliferation and immunoglobulin secretion of human B lymphocytes triggered through their CD40 antigen. J. Exp. Med. 173:705.

16. Korthauer, U., D. Graf, H.W. Mages, F. Brière, M. Padayachee, S. Malcolm, A.G. Ugazio, L.D. Notarangelo, R.J. Levinsky, and R.A. Kroczek. 1993. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. Nature (Lond.). 361:539.

17. Gilles, J.G., J. Arnoult, J. Vermylen, and J.M. Saint-Pryve. 1993. Anti-factor VIII antibodies of hemophilic patients are frequently directed towards nonfunctional determinants and do not exhibit isotypic restriction. Blood. 82:2452.

18. Banchereau, J., P. de Paoli, A. Vallé, E. Garcia, and F. Rousset. 1991. Long term human B cell lines dependent on interleukin 4 and anti-CD40. Science (Wash. DC). 251:70.

19. Rousset, F., E. Garcia, T. Defrance, C. Péronne, N. Vezzio, D.H. Hsu, R. Kastelein, K.W. Moore, and J. Banchereau. 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad. Sci. USA. 89:1890.

20. Splawski, J.B., and P.E. Lipsky. 1991. Cytokine regulation of immunoglobulin secretion by neonatal lymphocytes. J. Clin. Invest. 88:967.

21. Allen, R.C., R.J. Armitage, M.E. Conley, H. Rosenblatt, N.A. Jenkins, N.G. Copeland, M.A. Bedell, S. Edelhoff, C.M. Dice, D.K. Simoncini, et al. 1993. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science (Wash. DC). 259:990.

22. Aruffo, A., M. Farrington, D. Hollenbaugh, X. Li, A. Milatovich, S. Nonoyama, J. Bajorath, L.S. Grosmaire, K. Stenkamp, M. Neubauer, et al. 1993. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell. 72:291.

23. Mayer, L., D.N. Posnett, and H.G. Kunkel. 1985. Human malignant T cells capable of inducing an immunoglobulin class switch. J. Exp. Med. 161:134.

24. Te Velde, A.A., R. de Waal Malefyt, J.E. de Vries, and C.G. Figdor. 1992. IL-10 stimulates monocyte FcyR surface expression and cytotoxic activity. Distinct regulation of antibody-dependent cellular cytotoxicity by IFN-γ, IL-4 and IL-10. J. Immunol. 149:4048.