Interleukin 12 and CD86 regulate Th1 and Th2 development induced by a range of antigen doses presented by Peyer's patch and spleen cells

Tadashi Yoshida^{1,3}, Satoshi Hachimura^{1,*}, Mina Ishimori¹, Wataru Ise¹, Mamoru Totsuka¹, Akio Ametani² and Shuichi Kaminogawa¹

¹Department of Applied Biological Chemistry, The University of Tokyo, Tokyo 113-8657, Japan; ²Department of Immune Regulation, Torrey Pines Institute for Molecular Studies, San Diego, CA 92121-1122, USA;

³Present address: Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Fuchu 183-8509, Japan (*Author for correspondence: E-mail: ahachi@mail.ecc.u-tokyo.ac.jp; phone/fax: +81-3-5841-8029)

Received 29 December 2002; accepted in revised form 18 January 2003

Key words: antigen presentation, CD86, IL-12, Peyer's patch, Th1/Th2

Abstract

In this study, we demonstrate the role of interleukin 12 (IL-12), CD80 and CD86 in T helper type 1 (Th1) and Th2 differentiation induced through antigen presentation by Peyer's patch (PP) and spleen (SPL) cells with various doses of antigen. IL-12 was found to be critical for the induction of Th1-type cytokine producing cells, while antigen-dose dependent patterns of differentiation into Th2-type cytokine producing cells were not altered by the blockade of IL-12. Further, the difference in the pattern of Th2-type cytokine producing cell differentiation induced by PP and SPL cells depending on the antigen dosage were preserved in the absence of IL-12. When the function of CD86 was blocked by specific antibody, the induction of Th1-type cytokine producing cells was kept at high levels through every antigen dose, and the difference between PP and SPL cells was abrogated. With regard to Th2 induction, CD86 enhanced the differentiation of Th2-type cytokine producing cells but it was not essential in the case of antigen presentation by SPL cells. These results suggest that antigen-dose dependent changes in Th2 cell induction are regulated by additional factors which cannot induce antigen-dose dependent changes in Th1 cell differentiation by themselves.

Abbreviations: APC – antigen-presenting cell; DC – dendritic cell; IFN – interferon; IL – interleukin; OVA – chicken ovalbumin; PP – Peyer's patch; SPL – spleen; TCR – T cell receptor; Th – T helper

Introduction

In a previous study we showed that antigen presentation by PP and SPL cells induces both Th1 and Th2 T cell differentiation depending on the antigen dosage, and that the patterns were different between PP and SPL (Yoshida et al. 2002). In this report, we investigated the molecules

produced by PP and SPL cells which are involved in distinct Th1/Th2 differentiation induced by different antigen dosage.

It has been reported that the dosage of antigen influences many kinds of immune responses both *in vivo* and *in vitro* (Friedman and Weiner 1994; Constant et al. 1995; Hosken et al. 1995; Yoshida et al. 1997; Rogers and Croft 2000; Ruedl et al.

2000; Ise et al. 2002), and these effects on T cell differentiation have been most intensively investigated using T cell receptor (TCR) transgenic mice (TCR-Tg mice). In the previous reports, naive T cells from TCR-Tg mice differentiated into Th2 and Th1 by stimulation of low-dose and high-dose antigen, respectively (Constant et al. 1995). In addition, another group reported that still higher doses of antigen induced Th2 differentiation (Hosken et al. 1995). However, the mechanism of T cell differentiation induced by different doses of antigen are not yet clarified. Several *in vivo* experiments also indicated that the antigen dosage determines the direction of Th1/Th2 differentiation (Tsuji et al. 2001).

One mechanism of Th1 and Th2 differentiation by different doses of antigen that has been proposed is that the intensity of signals from the TCR alters intracellular signaling cascades. Therefore the influence of antigen-presenting cells (APCs) on such T cell differentiation has not been extensively investigated. Th1/Th2 differentiation induced by TCR-mediated signals was also reported using altered antigenic peptides (Boutin et al. 1997; Ise et al. 2000). In these reports, it was demonstrated that the substitution of one or a few amino acid residues of an antigenic peptide induced distinct T cell differentiation, and that the intracellular signaling of those T cells, such as the phosphorylation of CD3, was different from that of T cells stimulated by an agonistic peptide. However, recently several groups indicated the important roles of co-stimulatory signals via cytokines and/or cell surface molecules provided from APCs in Th1/Th2 differentiation induced by different doses of antigen (Rogers and Croft 2000). In addition, our previous study demonstrated that Th1 and Th2 induction by different doses of antigen showed distinct dependency on antigen doses through the antigen presentation by cells from different organs, Peyer's patches (PP) and spleen (SPL) (Yoshida et al. 2002). This observation strongly supports the roles of APCs in antigen-dose dependent T cell differentiation. Further, we found that these differences in Th1 and Th2 induction were not due to a simple shift of dosage, but distinct mechanisms gave rise to the unique dose dependent patterns of T cell differentiation. Together with these, it can be considered that cytokines and/or surface molecules expressed by PP and SPL cells were distinct and critical for T

cell differentiation induced by different doses of antigen. In this report, we investigated the molecules produced by PP and SPL cells which are involved in distinct patterns of Th1/Th2 differentiation induced by different antigen dosage. The roles of IL-12 and CD86 in the antigen-dose dependent changes of Th1 and Th2 induction were clearly demonstrated.

Materials and methods

Mice

Female BALB/c mice were purchased from Clea Japan (Tokyo, Japan) and used at 8–15 weeks of age. Chicken ovalbumin (OVA)-specific TCR transgenic mice (OVA 23–3) (Sato et al. 1994) were maintained by backcrossing to BALB/c mice. Tg T cells from these mice express $\alpha\beta$ -TCR that recognizes OVA_{323–339} restricted to I-A^d. Female TCR-Tg mice (8–20 weeks of age) were used in this study. All mice were maintained and used in accordance with the guidelines of the University of Tokyo.

Cell preparation

CD4⁺ T cells were purified from the splenocytes of TCR-Tg mice. Single-cell suspensions were labeled with anti-mouse CD4-conjugated magnetic microbeads and sorted with an LS column by magnetic cell sorting (MACS; Miltenyi Biotec, Bergish Gladbach, Germany). Although we purified CD62L high cells from these cells as naïve T cells and used them in preliminary experiments, we did not observe any difference from unfractionated CD4⁺ T cells (data not shown). Therefore, we show the data obtained with the whole CD4⁺ fraction of the splenocytes. PP cells and SPL cells were prepared from untreated BALB/c mice. PP and SPL cells obtained from these mice were washed several times in RPMI 1640 (Nissui Pharmaceutical, Tokyo, Japan) containing 100 U/ml of penicillin, 100 μ g/ml of streptomycin and 5×10^{-5} M 2-mercaptoethanol. Each of the organs was crushed with the end of a syringe, and single-cell suspensions were prepared by passing each cell suspension through a 200-mesh polyester filter (NBC Industry, Tokyo, Japan). The SPL

cells were treated with a modified ACT solution to deplete the erythrocytes.

Cell culture

PP or SPL cells from BALB/c mice were co-cultured with CD4⁺ T cells from TCR-Tg mice in RPMI medium. PP or SPL cells at 4×10^6 cells/well and CD4⁺ T cells at 1×10^6 cells/well were plated in 24-well (Coster, Cambridge, MA, U.S.A.) plates. OVA was added to the culture at the indicated concentrations. In this primary culture, the antibodies to IL-12 (C17.8, Genzyme, Cambridge, MA, U.S.A.), CD80 (RM80, (Nuriya et al. 1996) and CD86 (PO3, (Nuriya et al. 1996) were added at the concentration of $10 \mu\text{g/ml}$. The T cells were cultured for 1 week, collected from 24-well plates, washed and restimulated in 96-well plates at 1×10^5 cells/well with OVA at $2 \mu\text{M}$. In this secondary culture, irradiated or mitomycin-C treated SPL cells from BALB/c mice were used as APCs at 4×10^5 cells/ml. After 48 h of culture, the amounts of cytokines were measured by ELISA. For the IL-12 production assay, the supernatants were collected from wells without antibodies added at day 2 of the primary culture. All cultures contained 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD, USA).

Cytokine detection

The amounts of cytokines in the supernatants were measured by ELISA as previously described (Yoshida et al. 2002). In addition, we used rat anti-mouse IL-12 (C15.6, PharMingen, San Diego, CA, USA) and biotin-labeled rat anti-mouse IL-12 (C17.8, PharMingen) antibodies for IL-12 detection.

FACS analysis of CD80 and CD86

The cells were collected from the wells on the primary culture at day 1, and applied for FACS analysis. The cells were stained with biotin-labeled rat anti-mouse CD80 or CD86 antibodies, and streptavidin-Red670 (Gibco BRL). The cells were gated on the CD3 negative population by double

staining with FITC-labeled hamster anti-mouse CD3 antibody (145-2C11, Pharmingen).

Results

IL-12 is required for the differentiation into Th1-type cytokine producing cells but not for the induction of altered levels of Th2-type cytokine producing cells depending on antigen dose

Our previous report demonstrated that Th1/Th2 differentiation induced by various doses of antigen was differentially regulated in antigen presentation by PP and SPL cells. In the present study, we examined the mechanisms involved in such distinct antigen presenting ability of PP and SPL cells during the induction of Th1/Th2 differentiation by various doses of antigen. First, we focused on the role of IL-12 in the induction of Th1/Th2 cell development depending on antigen dosage. IL-12 is a critical cytokine for induction of Th1 differentiation. Naïve CD4⁺ T cells from TCR-Tg mice co-cultured with PP or SPL cells were differentiated into Th1- or Th2-type cytokine producing cells depending on antigen dose. The blockade of IL-12 by anti-IL-12 monoclonal antibody almost completely abrogated the differentiation of Th1-type cytokine producing cells induced by both PP and SPL cells (Figure 1). At $100 \mu\text{M}$, slight induction of IFN- γ producing cells was observed but it was substantially lower compared to that in the absence of anti-IL-12. On the other hand, the induction of Th2-type cytokine producing cells was enhanced in the presence of anti-IL-12 antibody, but the antigen-dose dependency was not significantly altered, except for IL-5. These results indicated that IL-12 is required for the induction of Th1 cells but not for the induction of distinct levels of Th2 cell differentiation depending on antigen doses. Interestingly, the amounts of IL-12 in the supernatants produced by PP and SPL cells did not correspond to the pattern of Th1/Th2 differentiation induced by various doses of antigen. IL-12 produced by both PP and SPL cells were simply increasing in a dose dependent manner and SPL cells produced higher amount of IL-12 than PP cells at any antigen concentration (Figure 2).

The effect of anti-IL-12 antibody on the differentiation of IL-5 secreting cells induced through

Figure 1. Effect of anti-IL-12 antibody on the development of Th1 and Th2 cells induced by PP (closed symbols) or SPL (open symbols) cells plus various concentrations of antigen. Naive $CD4^+$ T cells obtained from spleens of TCR-Tg mice were cultured with PP or SPL cells of BALB/c mice in the presence of various concentrations of OVA. Anti-IL-12 antibody was added to the culture at the beginning of the culture period (b). After 1 week, T cells were collected from each well and restimulated with APC and antigen as described in Materials and methods. Cytokine production in the supernatants from secondary cultures was determined by ELISA. The result is representative of three independent experiments.

Figure 2. IL-12 production induced by various doses of antigen. Naive $CD4^+$ T cells obtained from spleens of TCR-Tg mice were cultured with PP or SPL cells of BALB/c mice in the presence of various concentrations of OVA. After 2 days, culture supernatants were collected and the amounts of IL-12 were measured by ELISA. The result is representative of two independent experiments.

antigen presentation by PP cells was distinct from other Th2 cytokines. Although antigen presentation by SPL cells induced distinct levels of differentiation into IL-5 secreting cells depending on antigen-dose regardless of the presence or absence of IL-12, IL-5 induced by PP cells was simply increasing at antigen concentrations of 0.01–100 μ M (Figure 1b) in the absence of IL-12.

CD86 enhances the induction of Th2-type cytokine producing cells but is not required for distinct levels of Th2 cell induction depending on antigen dose

We next examined the role of co-stimulatory molecules, CD80 and CD86. The blockade of

Figure 3. Effect of anti-CD80 or CD86 antibody on the development of Th1 and Th2 cells induced by PP (closed symbols) or SPL (open symbols) cells plus various concentrations of antigen. Cells were cultured using methods described in Figure 1. Anti-CD80 (b) or CD86 (c) antibody was added to the culture and restimulation of T cells was performed. Cytokine production in the supernatants was determined and the result is representative of four independent experiments.

CD80 did not affect Th1/Th2 differentiation induced by PP and SPL cells (Figure 3b). On the other hand, the blockade of CD86 abrogated the antigen-dose dependency of differentiation into Th1-type cytokine producing cells induced by either PP or SPL cells (Figure 3a, c). The addition of anti-CD86 antibody enhanced the induction of IFN- γ secreting cells, especially in the case of antigen presentation by SPL cells, at antigen concentrations where low levels of IFN- γ producing cells was induced, and the difference in the pattern induced by PP and SPL cells was abrogated. Although the blockade of CD86 down-regulated induction of Th2-type cytokine producing cells by both PP and SPL cells, IL-4, IL-5 and IL-10 secreting cells were still observed with similar antigen-dose dependency to those observed before the blockade in the case of SPL cells

(Figure 3a, c). These results indicated that antigen-dose dependent induction of Th1- and Th2-type cytokine producing cells are independently regulated and there might be other factors in the system which determine the distinct levels of Th2 cell induction depending on antigen dose.

The expression of CD80 and CD86 on CD3 negative populations of PP and SPL cells stimulated with various doses of antigen, collected from the primary culture, was also examined. CD86 expression on both PP and SPL cells was found to increase depending on the antigen dosage, although SPL cells expressed higher levels of CD86 than PP cells at any antigen concentration (Figure 4b). This result showed that the expression of CD86 did not correspond to the pattern of Th1/Th2 cell induction. This also suggests that the distinct patterns of antigen-dose dependent

Figure 4. Expression of CD80 and CD86 induced by various doses of antigen on CD3-negative PP and SPL cells. Naive CD4⁺ T cells obtained from spleens of TCR-Tg mice were cultured with PP or SPL cells from BALB/c mice in the presence of various concentrations of OVA. After twenty-four hours, the cells were collected from each well and the expression of CD80 (a) and CD86 (b) was detected as described in Materials and methods. The result shows the median fluorescence intensity (MFI) of CD3-negative cells and the result is representative of two independent experiments.

Th1/Th2 cell differentiation may be regulated by other molecules. The expression of CD80 on PP and SPL cells was not altered by antigen stimulation at any concentration (Figure 4a).

Discussion

Ruedl et al. reported that T cells stimulated by high-dose antigen express high levels of CD40L, and that these CD40L induce IL-12 production by dendritic cells (DCs) and macrophages, which plays a critical role in Th1 differentiation induced by various doses of antigen (Ruedl et al. 2000). Therefore, in this study, we examined the role of IL-12 by *in vitro* blocking experiments using anti-IL-12 monoclonal antibody. Our results showed that the induction of IFN- γ secreting cells, indicating Th1 induction, was almost completely inhibited by anti-IL-12 antibody when using either PP or SPL cells as APCs. This observation demonstrates that IL-12 is critical for Th1 induction

regardless of the type of APC. On the other hand, Th2-type cytokines, IL-4, IL-5 and IL-10, were induced depending on antigen dosage, and the dose-dependent kinetics still differed between those induced by PP and SPL cells, even in the presence of anti-IL-12 antibody. This result demonstrates that IL-12 is not necessary for the induction of antigen-dose dependent changes of Th2 differentiation. Moreover, the production of IL-12 increased depending on antigen dose and the amount of IL-12 produced by SPL cells was higher than that produced by PP cells at any given dose. Together with these results, our findings showed that the pattern of IL-12 production did not contribute to Th1/Th2 differentiation induced by various doses of antigen, indicating that other factors are likely to act to induce low levels of Th2 differentiation.

Next, we examined the function of CD80 and CD86 in Th1/Th2 differentiation induced by various antigen doses. CD80 and CD86 are considered to play critical roles in the activation and differentiation of T cells by functioning as co-stimulatory molecules. The blockade of CD86 by the addition of anti-CD86 antibody abrogated the antigen-dose dependency of Th1 differentiation. Thus, antigen presentation by PP and SPL cells induced similar Th1 differentiation by blocking CD86 function. Blocking CD86 enhanced Th1 induction especially when strong Th2 induction was observed i.e., SPL cells plus 0.1–1 μ M OVA and PP cells plus 10 μ M OVA. In contrast, the blockade of CD86 inhibited the induction of Th2 differentiation. These observations suggest that CD86 is simultaneously involved in reduced induction of Th1 and enhanced induction of Th2. Nevertheless, antigen presentation by SPL cells induced weak Th2 differentiation even after CD86 was blocked and the antigen-dose dependency of Th2 induction did not change. These results indicate that CD86 enhances Th2 differentiation but is not essential for Th2 induction. Together with these results, it is suggested that other factors would regulate differentiation into Th2 cells, which is induced by various doses of antigen. Although it is uncertain that such factors would work in antigen presentation by PP cells, since Th2 differentiation was almost inhibited by the blockade of CD86, we considered that similar factors may also be involved in Th2 induction by antigen presentation of PP cells. This is because the expression

pattern of CD86 on PP cells did not correspond with the pattern of Th2 induction by various doses of antigen. The function of CD86 in Th1/Th2 differentiation is controversial although several reports have suggested that CD86 enhances Th2 differentiation (Freeman et al. 1995; Ranger et al., 1996). Our observations also show that CD86 enhances Th2 differentiation induced through antigen presentation by PP and SPL cells. Such Th2 enhancing ability of CD86 may inhibit Th1 induction. Thus, the abrogation of dose dependency in Th1 induction by blocking CD86 may not be due to a direct effect on Th1 induction but rather an indirect effect via inhibition of Th2 induction.

The expression of CD80 did not differ between PP and SPL cells, and did not change over a range of antigen concentrations. Moreover, the blockade of CD80 barely affected T cell differentiation. On the other hand, we confirmed the activity of the anti-CD80 antibody by another experiment. The blockade of CD86 alone did not inhibit the T cell proliferative response but simultaneous blockade of CD80 and CD86 was completely inhibitory (data not shown). This observation suggests that CD80 does not affect Th1/Th2 differentiation induced by various doses of antigen when CD86 coexists. Conversely, although CD80 provided a co-stimulatory signal in the absence of CD86, the antigen-dose dependent T cell differentiation induced by CD80 was different from those induced by CD86. However, it was possible that the activity of the anti-CD80 antibody was insufficient to detect the change of CD80 expression in our system.

Interestingly, the blockade of IL-12 did not alter the antigen dose dependency of induction of all Th2 type cytokines, IL-4, IL-5 and IL-10, in the case of antigen presentation by SPL cells, while IL-5 production was simply increasing depending on antigen dose when PP cells were used. This phenomenon was observed only in IL-5 production, and dose dependency of other cytokines, IL-4 and IL-10, did not change. This result demonstrates that IL-5 is regulated differentially from other Th2 type cytokines and the regulatory system differs entirely between PP and SPL cells. One possibility is that high levels of IL-5 could be induced only by PP cells but IL-12 produced by some PP cells inhibits the induction at high doses of antigen. Another possibility is that both PP and SPL cells express a factor which inhibits IL-5 production at

high antigen doses in the presence of IL-12, while PP cells could not express such factor in the absence of IL-12. PP is an inductive site of mucosal immune responses and is considered a critical organ for the generation of IgA producing cells (Spalding et al. 1984; George and Cebra, 1991). The distinct ability of PP cells for IL-5 induction found in this study may be involved in IgA induction *in vivo*.

In this study, PP and SPL cells were used as whole populations without further fractionation into individual cell populations since we intended to reconstitute, as far as possible, an *in vivo* response. We consider that our system represented several factors involved in *in vivo* responses, such as cytokines and surface molecules, although it is not complete because the three dimensional structure of the organ was disrupted during cell preparation. However, it could not be clarified which cell population induces such T cell differentiation in our system, although it is considered to be important because several recent reports demonstrated that various types of DCs exist in PP and induce distinct T cell responses (Iwasaki and Kelsall 1999, 2000, 2001). We also fractionated B and non-B cells from PP and SPL cells and used them as APCs to examine their antigen presenting ability for the induction of Th1/Th2 differentiation. Our observation demonstrated that a mixture of each population from these organs induced different T cell differentiation patterns from those induced by individual populations (unpublished observations). Moser reported that CD86 expressed on macrophages induced Th2 differentiation, while expression on DCs induced Th1 (Moser 2001). This finding indicates that the apparent opposing functions of CD86 are dependent on other coexisting factors. As described above, our results showed that CD86 enhanced Th2 induction by both PP and SPL cells. However, this effect may change if these cells are used after further fractionation. Together with these, it is considered that the antigen-dose dependency of Th1/Th2 differentiation may be altered when different types of cells are involved in antigen presentation. We would therefore like to examine the specific antigen-dose dependency of Th1/Th2 differentiation induced by PP and SPL cells *in vivo*.

In this study, our results demonstrated that Th1/Th2 differentiation induced by various antigen doses is regulated in a complex manner by various

factors which are produced by cells coexisting with T cells. This finding suggests that a certain antigen may induce Th1/Th2 differentiation with different dose-dependency when it encounters T cells at different sites.

Acknowledgements

We thank Drs. Sonoko Habu, Takehito Sato (Tokai University School of Medicine, Isehara) and Yoshihiro Kumagai (Nippon Medical School, Tokyo) for generously presenting the TCR-transgenic mice. We are also grateful to Drs. K. Okumura and H. Yagita (Juntendo University) for kindly providing the anti-CD80 (RM80) and CD86 (PO3) antibodies.

References

- Boutin Y., Leitenberg D., Tao X. and Bottomly K. 1997. Distinct biochemical signals characterize agonist- and altered peptide ligand-induced differentiation of naive CD4⁺ T cells into Th1 and Th2 subsets. *J. Immunol.* 159: 5802–5809.
- Constant S., Pfeiffer C., Pasqualini T. and Bottomly K. 1995. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4⁺ T cells. *J. Exp. Med.* 182: 1591–1596.
- Freeman G.J., Boussiotis V.A., Anumanthan A., Bernstein G.M., Ke X.Y., Rennert P.D., Gray G.S., Gribben J.G. and Nadler L.M. 1995. B7-1 and B7-2 do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. *Immunity* 2: 523–532.
- Friedman A. and Weiner H.L. 1994. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc. Natl. Acad. Sci. USA* 91: 6688–6692.
- George A. and Cebra J.J. 1991. Responses of single germinal-center B cells in T-cell-dependent microculture. *Proc. Natl. Acad. Sci. USA* 88: 11–15.
- Hosken N.A., Shibuya K., Heath A.W., Murphy K.M. and O'Garra A.O. 1995. The effect of antigen dose on CD4⁺ T helper cell phenotype development in a T cell receptor- $\alpha\beta$ -transgenic model. *J. Exp. Med.* 182: 1579–1584.
- Ise W., Totsuka M., Takato R., Hachimura S., Sato T., Ametani A., Kumagai Y., Habu S. and Kaminogawa S. 2000. Primary response of naive CD4⁺ T cells to amino acid-substituted analogs of an antigenic peptide can show distinct activation patterns: Th1- and Th2-type cytokine secretion, and helper activity for antibody production without apparent cytokine secretion. *FEBS Lett.* 465: 28–33.
- Ise W., Totsuka M., Sogawa Y., Ametani A., Hachimura S., Sato T., Kumagai Y., Habu S. and Kaminogawa S. 2002. Naive CD4⁺ T cells exhibit distinct expression patterns of cytokines and cell surface molecules on their primary responses to varying doses of antigen. *J. Immunol.* 168: 3242–3250.
- Iwasaki A. and Kelsall B.L. 1999. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J. Exp. Med.* 190: 229–239.
- Iwasaki A. and Kelsall B.L. 2000. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3 α , MIP-3 β , and secondary lymphoid organ chemokine. *J. Exp. Med.* 191: 1381–1394.
- Iwasaki A. and Kelsall B.L. 2001. Unique functions of CD11b⁺, CD8 α ⁺, and double-negative Peyer's patch dendritic cells. *J. Immunol.* 166: 4884–4890.
- Moser M. 2001. Regulation of Th1/Th2 development by antigen-presenting cells *in vivo*. *Immunobiol.* 204: 551–557.
- Nuriya S., Yagita H., Okumura K. and Azuma M. 1996. The differential role of CD86 and CD80 co-stimulatory molecules in the induction and the effector phases of contact hypersensitivity. *Int. Immunol.* 8: 917–926.
- Ranger A.M., Das M.P., Kuchroo V.K. and Glimcher L.H. 1996. B7-2 (CD86) is essential for the development of IL-4-producing T cells. *Int. Immunol.* 8: 1549–1560.
- Rogers P.R. and Croft M. 2000. CD28, Ox-40, LFA-1, and CD4 modulation of Th1/Th2 differentiation is directly dependent on the dose of antigen. *J. Immunol.* 164: 2955–2963.
- Ruedl C., Bachmann M.F. and Kopf M. 2000. The antigen dose determines T helper subset development by regulation of CD40 ligand. *Eur. J. Immunol.* 30: 2056–2064.
- Sato T., Sasahara T., Nakamura Y., Osaki T., Hasegawa T., Tadakura T., Arata Y., Kumagai Y., Katsuki M. and Habu S. 1994. Naive T cells can mediate delayed-type hypersensitivity response in T cell receptor transgenic mice. *Eur. J. Immunol.* 24: 1512–1516.
- Spalding D.M., Williamson S.I., Koopman W.J. and McGhee J.R. 1984. Preferential induction of polyclonal IgA secretion by murine Peyer's patch dendritic cell-T cell mixtures. *J. Exp. Med.* 160: 941–946.
- Tsuji N.M., Mizumachi K. and Kurisaki J. 2001. IL-10 secreting Peyer's patch cells are responsible for active suppression in low-dose oral tolerance. *Immunology* 103: 458–464.
- Yoshida T., Hachimura S. and Kaminogawa S. 1997. The oral administration of low-dose antigen induces activation followed by tolerization, while high-dose antigen induces tolerance without activation. *Clin. Immunol. Immunopathol.* 82: 207–215.
- Yoshida T., Hachimura S., Ishimori M., Kinugasa F., Ise W., Totsuka M., Ametani A. and Kaminogawa S. 2002. Antigen presentation by Peyer's patch cells can induce both Th1- and Th2-type responses depending on antigen dosage, but a different cytokine response pattern from that of spleen cells. *Biosci. Biotechnol. Biochem.* 66: 963–969.