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REVIEW ARTICLE

Adjuvants that Enhance Th2 or Tr Responses

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ABSTRACT

It is well-known that many isoforms of toll-like receptors (TLRs) function as Th1 adjuvant receptors. Thus, the ligands induce Th1 differentiation in an antigen non-specific manner. During the past few years, not only Th1, but also Th2 adjuvants have been reported. Allergy-inducing materials, such as parasites, first stimulate dendritic cells (DCs) to change their character as professional antigen-presenting cells. Such a DC population (DC2) can stimulate naive CD4T cells to induce differentiation into Th2. In some instances, DCs that can stimulate regulatory T cells are also induced. Interestingly, many of such substances are glycolipids or phospholipids that mammalian species do not usually carry. In this paper, we show a cellular and molecular basis for Th2 adjuvants.

KEY WORDS

adjuvants, allergy, dendritic cells, HLA, Notch ligands, Th2, toll-like receptors

INTRODUCTION

Recent progress in dendritic cell (DC) research revealed that DCs with a specific character play pivotal roles in inducing Th2 responses. Moreover, allergy-inducing substances, carry not only protein allergen but also other biological activities that relate to allergy. Most of these activities are carried by small molecules with adjuvant properties. DCs are usually located in the interface between such environment and the human body. In this sense, DCs play distinct roles, compared with T cells and B cells. Thus, adjuvant activities affect DCs to exert allergen non-specific induction of allergic responses.

TH1 ADJUVANTS

Toll-like receptor (TLR) molecules expressed on DCs function as adjuvant receptors.[1] Environmental molecules, such as certain nucleic acids,[2,3] lipopolysaccharides (LPS),[4] and fungus-derived glycoprotein molecules,[5] alter DC function, by binding with a certain isoform of TLR, and they usually induce Th1 responses. Because such DCs induce Th1 responses, they are designated DC1, and such environmental molecules are called Th1 adjuvants (Fig. 1).

TH2 ADJUVANTS

DC2 and Th2 adjuvants have been recently reported. Th2 adjuvants induce Th2 responses in an antigen non-specific manner, through the induction of DC2. In other words, DCs that stimulate Th2 differentiation are called DC2. Whelan *et al.* reported in 2000 the Th2 adjuvant ES-62.[6] ES-62 is a phosphorylcholine-containing glycoprotein derived from nematode of filaria. Bone marrow-derived immature DCs were first incubated with GM-CSF and ES-62 for 24 hours, followed by co-incubation with naive CD4T cells of DO11.10-Tg mice, in the presence of OVA peptide. Activated T cells were then re-stimulated with PMA + ionomycin, and their cytokine production levels were determined. Indeed, ES-62 apparently increased the IL-4/IFN-γ ratio, without affecting CD80/86 balance.

van der Kleij *et al.* reported in 2002 that schistosoma-derived phosphatidylserine directly stimulates DCs and leads to Th2 induction (Fig. 2). Interestingly, neither synthetic nor mammalian phosphatidylserine has such an activity. Schistosoma-derived phosphatidylserine supposedly exhibits such an activity through a unique structure in its acyl base.[7] In other words, parasites carry "allergens", because they also carry Th2 adjuvant activity in an antigen non-specific manner.

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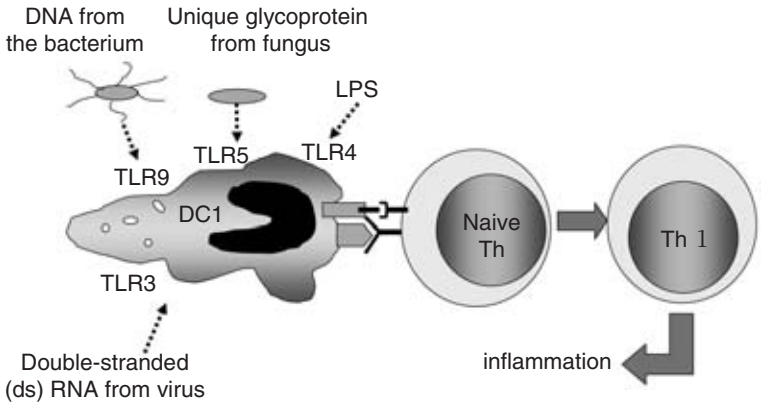

Fig. 1 Th1 adjuvant. DCs stimulated with pathogen-associated molecular patterns (PAMPs) derived from microbes induce differentiation of naive T cells into Th1 cells. TLR3, TLR7, TLR8 and TLR9 exist in the endosomes, while the other TLR isoforms are expressed on the plasma membrane.

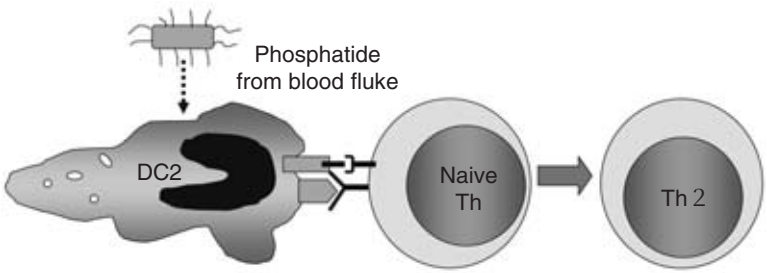

Fig. 2 Th2 adjuvant. DCs stimulated with phospholipids derived from helminth induce differentiation of naive T cells into Th2 cells.

Schistosomas do not synthesize fatty acids *de novo*. They modify the structure of host-derived fatty acids, by using their own enzymes to elongate the acyl base, thus allowing the appearance of parasite-specific fatty acids, in the host-parasite interface. Interestingly, unsaturated lysophosphatidylserine (lyso-PS) with a single acyl base, tends to modify DC function to induce IL-10-producing regulatory T cells (Tr). This is partially blocked by a neutralizing antibody to TLR2. On the other hand, Th2-inducing phosphatidylserine with two acyl bases does not appear to use TLR2 (Fig. 3).[8,9] Lysophosphatidic acid (Lyso-PA) and sphingosine-1-phosphate (S1P) also induce DC2, whereas lysophosphatidylcholine (Lyso-PC) induces DC1. Because they are structurally similar in their acyl bases, head groups might be important in determining their activity (Fig. 4).

It is yet to be determined as to what molecules on DCs regulate T-cell differentiation. CCL2 or monocyte chemoattractant protein-1 (MCP-1) are produced by DCs. Gu *et al.* reported that in CCL2-/- mice, decreased levels of Th2 and IgG1 responses to TNP-OVA are observed. Indeed, they become resistant to leishmania infection.[10] However, CCL2 activity in humans has not yet been delineated. Certain immunity-related molecules such as thymic stromal lymphopoietin (TSLP) are differentially expressed between humans and mice, raising the possibility that rules in mice do not apply to humans.

Prostaglandins (PG), especially, PGE2 and PGD2 have been reported to carry Th2 adjuvant activity. In this concern, Kalinski *et al.* reported that immature DCs produce IL-12p40, when stimulated with TNF-α and PGE2. Indeed, monomeric or homodimeric p40 act as antagonists for IL-12p70, allowing PGE2 to function as a Th2 adjuvant. However, this is not the only one mechanism by which PGE2 functions as a Th2 adjuvant. Other mechanisms include: 1) PGE2 acts directly on T cells and suppress IFN-γ production; 2) PGE2 inhibits IL-12R expression; and 3) PGE 2 stimulates monocytes to produce IL-10.[11] Another topic in this concern is a recent observation that pollen carries molecules cross-reactive with human PGE 2 or leukotriens.[12]

Other Th2 adjuvants include molecules expressed by *C. albicans*, histamine, G protein-R, PRR glycolipids, OX40L, Jagged 1, and Pam3Cys-Ser-Lys4 (Pam3Cys). Pam3Cys is an active moiety of

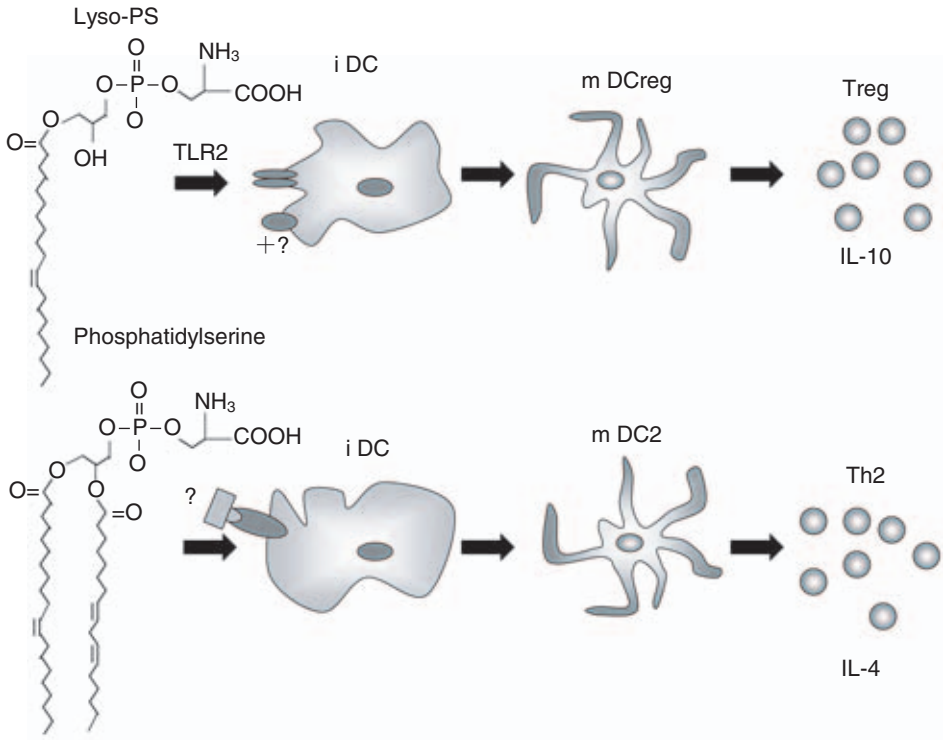

Fig. 3 Structure of Lyso-PS and phosphatidylserine which induce regulatory T cells and Th2 cells respectively.

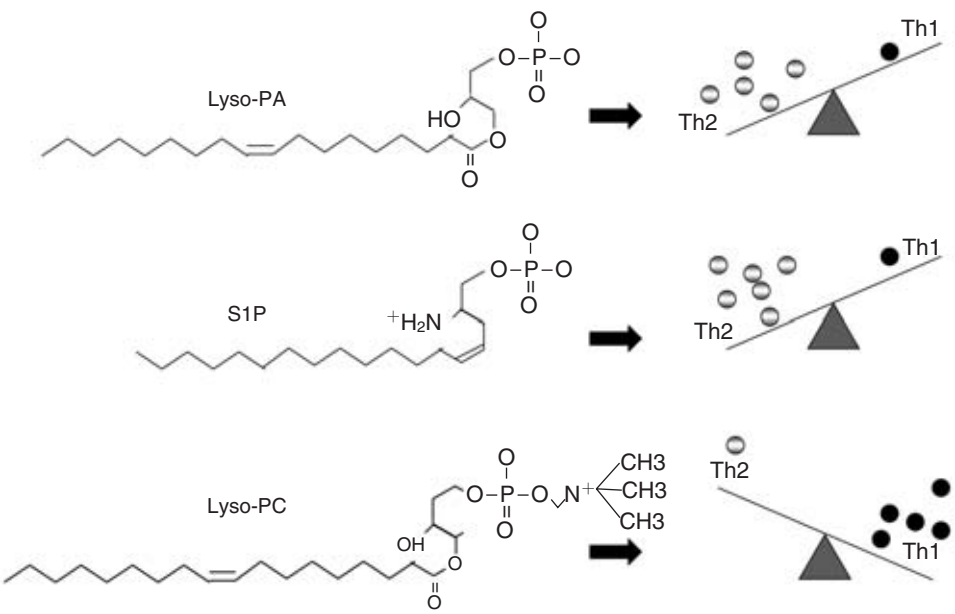

Fig. 4 Structural differences in Lyso-PC, S1P, and Lyso-PC.

mycoplasma-associated lipopeptide 2, and leads to the activation of Erk and the stabilization of c-Fos.[9,13]

Then, is it necessary for Th2 adjuvants to reach secondary lymphatic organs, to exhibit their biological activity? In this concern, Coker *et al*. reported that a genealogical tree can be drawn by using VH hypermutation obtained from the nasal mucosa of allergic rhinitis patients. These mutations include very similar VDJ families of IgE and IgA classes, which are highly indicative of local somatic hypermutation. Further-

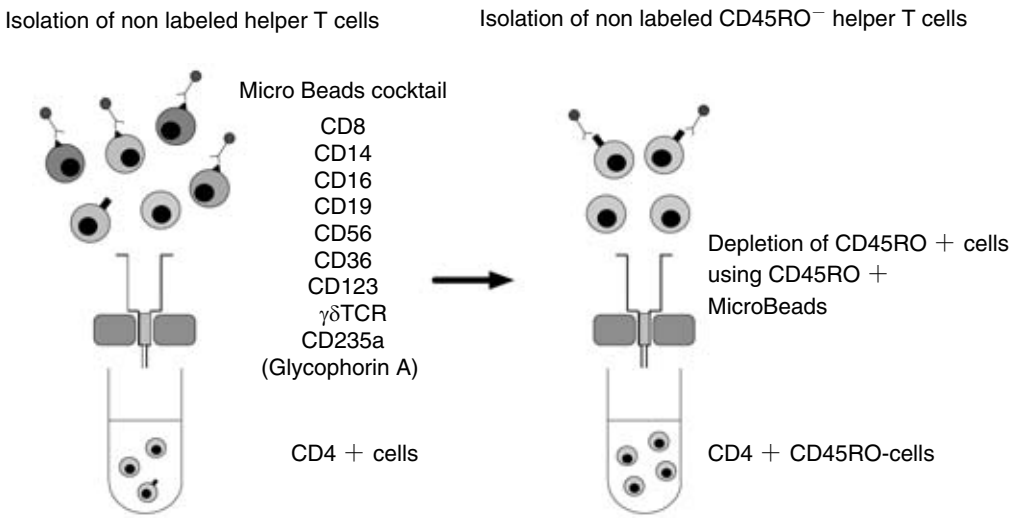

Fig. 5 Preparation of naive CD4 T cells by magnetic separation.

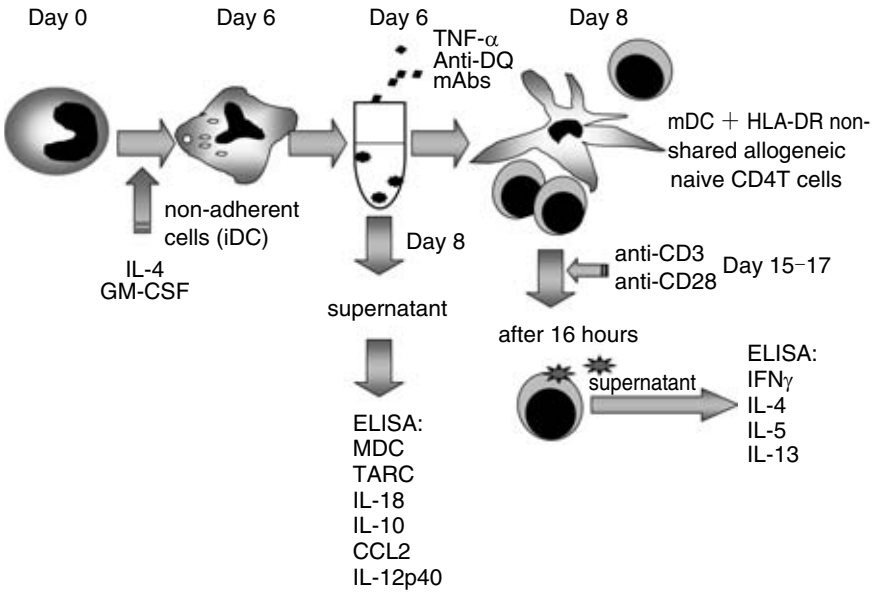

Fig. 6 Overview of the assay protocol for determination of the adjuvant activity.

more, activation-induced cytidine deaminase (AID) activity was increased *in situ*. None of these observations apply for allergic patients without rhinitis, indicating that even the periphery is a place for somatic hypermutation and class switching.[14]

With regard to local reactivities, Soumelis *et al*. reported an interesting phenomenon.[15] TSLP is an IL-7-like cytokine. Murine TSLP plays a pivotal role in primary T/B differentiation, but not in DCs. On the other hand, in humans, TSLP activates CD11c-positive DCs but has no effect on T/B differentiation, because TSLP receptor molecules are expressed on DCs but not on T/B cells in humans. They stimulated DCs with TSLP, LPS and CD40L for 24 hours, followed by the induction of alloreactive T cell responses using naive CD4 T cells. Six days after primary stimulation, T cells were re-stimulated with anti-CD3 + anti-CD28, followed by the determination of cytokine levels produced by T cells. Interestingly, TSLP-stimulated DCs produced TARC and MDC, and induced T-cell differentiation towards Th2 type.

TSLP molecules are expressed solely on mast cells in bone marrow-derived cells, and in other tissues such as lung fibroblasts, bronchial smooth muscle cells, skin keratinocytes, etc. Indeed, in atopic dermatitis patients, DC-LAMP-positive DCs are co-localized with TSLP-positive keratinocytes. It may be that environmental substances stimulate the skin to produce

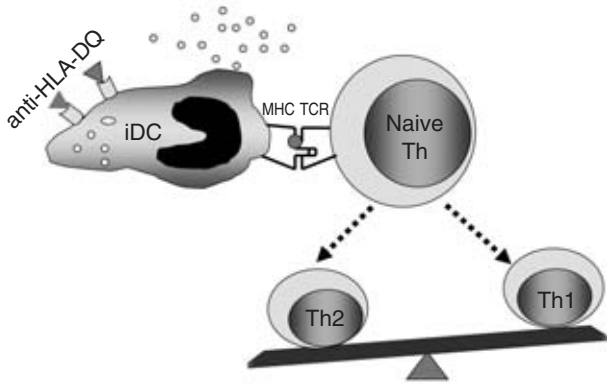

Fig. 7 Stimulation of HLA-DQ induces the differentiation of DC into DC2. HLA-DQ-mediated signals are transmitted, as described below. 1. Anti-HLA-DQ Ab. 2. Biotinylated anti-HLA-DQ Ab plus avidin. 3. Plate-bound anti-HLA-DQ Ab. 4. Agarose-conjugated anti-HLA-DQ Ab. 5. Emetine (*de novo* protein synthesis inhibitor) treated and HLA-DQ restricted T cells.

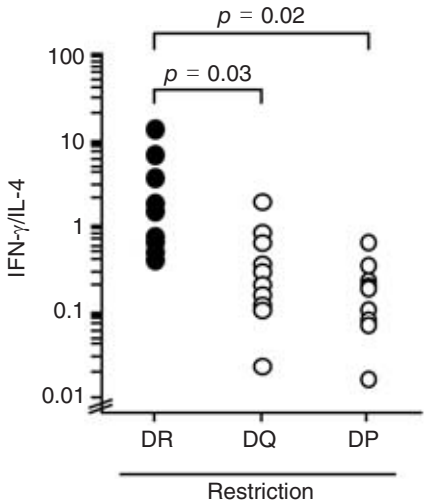

Fig. 8 Restriction molecules and the differentiation of Th1/2. HLA-DR-, DQ-, and DP-restricted T cells responsible for 19-mer peptide with random sequences (X19) were stimulated by 500 μM X19 with antigen-presenting cells. After 48-hour incubation, culture supernatants were collected for measurements of IFN-γ and IL-4 production by ELISA. One spot indicates one cell line.

TSLP, which activates DCs to produce Th2-attracting chemokines. These DCs move towards lymph nodes where they stimulate allergen-specific Th2 cells, which in turn migrate back to the skin, based on the concentration gradient of TARC/MDC. All these observations indicate that keratinocytes play a key role between the environment and DCs. It is conceivable that mast cells and NKT cells also play a role between the environment and DCs.

THE ASSAY SYSTEM FOR TH2 ADJUVANTS

We have been working under the hypothesis that allergy-inducing substances such as food, endocrine disruptors, mites, and pollen may also carry Th2 adjuvant activity. Indeed, some of these substances were shown to carry such an activity, through our studies. Briefly, CD14-positive cells were isolated from human PBMCs and incubated with IL-4 and GM-CSF for 6 days. These cells (immature DCs) were re-stimulated with TNF-α, in the presence of adjuvants to be tested. Two days after the incubation with adjuvants, supernatants were collected for the determination of IL-12p 40, MDC, TARC, CCL-2 and IL-10. Cellular components were further co-cultured with HLA-DR-non-shared allogeneic naive CD4T cells (Fig. 5) to induce mixed lymphocyte reaction (MLR). This process allows naive T cells to differentiate into Th1/Th2. Seven to nine days later, differentiated T cells were re-stimulated with agonistic anti-CD3 and anti-CD28 antibodies, to be subjected to supernatant collection for the determination of IL-4/IFN-γ (Fig. 6).

ANTIGEN-PRESENTING MOLECULES AND ADJUVANT ACTIVITY

We have been working with signal transduction mechanisms through HLA molecules, and reported that class II isoforms (DR, DQ and DP) transmit distinct signals into antigen-presenting cells (APCs). When HLA-DQ and DP are used for antigen presentation, crosslinking of HLA-DQ and DP induced anti-inflammatory monokine production from monocytes.[16] To further address these issues using DCs, we stimulated DCs with anti-HLA-DQ mAb, and found that signaling through HLA-DQ can induce differentiation of DC2 (Fig. 7).[17] We then established short-term T-cell lines reactive to randomized 19-mer peptide (X19), determined restriction molecules clone by clone, and tested how Th1/Th2 shift patterns are associated with restriction HLA molecules (Fig. 8). Indeed, as expected, HLA-DQ- and DP-restricted T cells tend to show a Th2-shifted phenotype as compared with DR-restricted ones. These observations confirmed our previous findings on HLA-linked immune-suppression genes,[18] at cellular levels.

NOTCH LIGANDS AND ADJUVANT ACTIVITIES

Notch signaling pathways are highly conserved in organisms ranging from invertebrates to mammals and involved in cell fate choice during development. This signaling is caused by interactions between the Notch receptors and the Notch ligands, Delta and Jagged members.[19] In mammals, four Notch receptors (Notch 1-4) and five Notch ligands (Delta 1,

Delta3, Delta4, Jagged1, and Jagged2) have been identified. Notch signaling has been shown to play a critical role in several steps of lymphoid cell commitment and lineage decision.[20]

Recently, Amsen *et al.* have presented evidence that different Notch ligands induced by Th1/Th2 adjuvant activities on APCs instruct mature T cell differentiation in mice.[21] In their *in vitro* study, LPS known as a strong Th1 adjuvant induced Delta4 and Jagged1 expression on DCs. MyD88-dependent TLR4 signaling was required for the induction of Delta4 expression, while the Jagged1 expression was up-regulated through MyD88-independent TLR4 signaling. LPS can promote not only Th1 but also Th2 responses under some conditions.[22] Th1 responses induced by LPS depend on MyD88, whereas a MyD88-independent pathway promotes Th2 responses.[23] Thus, Jagged1 expression correlates with the ability of LPS to promote Th2 responses, while Delta4 expression correlates with the ability of LPS to promote Th1 responses. On the other hand, Th2 adjuvants, PGE2 and cholera toxin induced Jagged2 expression, whereas expression of Jagged1 and all Delta members was barely affected.[21] APC retrovirally transduced to express Jagged1 promoted Th2 differentiation, while APC retrovirally transduced to express Delta1 caused Th1 polarization and reduced Th2 responses.[21] Based on these observations, Amsen *et al.* concluded that the Jagged expression induced by Th2 adjuvants on APCs contributes to Th2 polarization while the Delta expression induced by Th1 adjuvants on APCs contributes to Th1 polarization. Consistent with their conclusion, Maekawa *et al.* have demonstrated induction of mouse Th1 differentiation by Delta1-Notch3 interactions partly independent of IL-12 signaling *in vitro*.[24]

However, more recently, de La Coste *et al.* have presented a possibility that Delta4 may play a role in induction of Th2 but not Th1 responses, reconstituting the immune system of lethally irradiated athymic nu/nu mice with fetal liver cells retrovirally transduced to overexpress Delta1 or Delta4.[25] In addition, several studies suggest that Jagged1 induces Ag-specific Tr differentiation instead of Th2 differentiation.[26-28]

Collectively, Notch ligands expressed on APCs potentially impact Th1/Th2/Tr differentiation, but the nature of such effects still remains obscure. Most of the previous studies have been based on mouse experiments. To assess whether and how Th1/Th2 adjuvant activities affect Notch ligand expression on human APCs, our efforts are underway using several types of APC, such as monocyte-derived DCs and several cell lines of monocytic or DC-like lineage. In the preliminary experiments, Th1/Th2 adjuvants indeed altered Notch ligand expression profiles on human APCs, although the patterns were partly different from those previously observed in mice.[21] In addition, the alteration in Notch ligand expression varied to some degree among different types of APC (unpublished data).

REFERENCES

1. Takeda K, Akira S. Toll receptors and pathogen resistance. *Cell Microbiol.* 2003;**5**:143-153.
2. Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annu. Rev. Immunol.* 2002;**20**:709-760.
3. Matsumoto M, Kikkawa S, Kohase M, Miyake K, Seya T. Establishment of a monoclonal antibody against human Toll-like receptor 3 that blocks double-stranded RNA-mediated signaling. *Biochem. Biophys. Res. Commun.* 2002; **293**:1364-1369.
4. Fitzgerald KA, Palsson-McDermott EM, Bowie AG *et al.* Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. *Nature* 2001;**413**:78-83.
5. Ozinsky A, Underhill DM, Fontenot JD *et al.* The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc. Natl. Acad. Sci. U.S.A.* 2000;**97**: 13766-13771.
6. Whelan M, Harnett MM, Houston KM, Patel V, Harnett W, Ringley KP. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J. Immunol.* 2000;**164**:6453-6460.
7. van der Kleij D, Latz E, Brouwers JFHM *et al.* A novel host-parasite lipid cross-talk. *J. Biol. Chem.* 2002;**50**: 48122-48129.
8. van der Kleij D, Yazdanbakhsh M. Control of inflammatory diseases by pathogens: lipids and the immune system. *Eur. J. Immunol.* 2003;**33**:2953-2963.
9. Redecke V, Haecker H, Datta SK *et al.* Activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J. Immunol.* 2004;**172**:2739-2743.
10. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of Th2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* 2000;**404**:407-411.
11. Kalinski P, Vieira PL, Schuitemaker JHN, de Jong EC, Kapsenberg ML. Prostaglandin E2 is a selective inducer of interleukin-12 p40 (IL-12p40) production and an inhibitor of bioactive IL-12 p70 heterodimer. *Blood* 2001;**97**: 3466-3469.
12. Traidl-Hoffmann C, Kasche A, Menzel A *et al.* Impact of pollen on human health: More than allergen carriers? *Int. Arch. Allergy Immunol.* 2003;**131**:1-13.
13. Agrawal S, Agrawal A, Doughty B *et al.* Different toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J. Immunol.* 2003;**171**:4984-4989.
14. Coker HA, Durham SR, Gould HJ. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J. Immunol.* 2003;**171**: 5602-5610.
15. Soumelis V, Reche PA, Kanzler H *et al.* Human epithelial cells trigger dendritic cell-mediated allergic inflammation by producing TSLP. *Nature Immunol.* 2002;**3**:673-680.
16. Matsuoka T, Tabata H, Matsushita S. Monocytes are differentially activated through HLA-DR, -DQ and -DP molecules via MAP kinases. *J. Immunol.* 2001;**166**:2202-2208.
17. Matsushita S, Liu T-Y, Ohyama H, Uemura Y. Signal transduction through HLA-DQ molecules alter dendritic cell function to enhance Th2 differentiation. EAACI;2004, Am-

sterdam (abstract).
18. Hirayama K, Matsushita S, Kikuchi I, Iuchi M, Ohta N, Sasazuki T. HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. *Nature* 1987;**327**:426-430.
19. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science* 1999;**284**:770-776.
20. Maillard I, Adler SH, Pear WS. Notch and the immune system. *Immunity* 2003;**19**:781-791.
21. Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction of CD4 T helper cell fates by different Notch ligands on antigen-presenting cells. *Cell* 2004; **117**:515-526.
22. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* 2002;**196**:1645-1651.
23. Kaisho T, Hoshino K, Iwabe T, Takeuchi O, Yasui T, Akira S. Endotoxin can induce Myd88-deficient dendritic cells to support T (h) 2 cell differentiation. *Int. Immunol.* 2002;**14**:695-700.
24. Maekawa Y, Tsukumo S, Chiba S *et al.* Delta1-Notch3 interactions bias the functional differentiation of activated CD4+ T cells. *Immunity* 2003;**19**:549-559.
25. de La Coste A, Six E, Fazilleau N *et al. In vivo* and in absence of a thymus, the enforced expression of the Notch ligands Delta-1 or Delta-4 promotes T cell development with specific unique effects. *J. Immunol.* 2005;**174**:2730-2737.
26. Hoyne GF, Le Roux I, Corsin-Jimenez M *et al.* Serrate 1-induced notch signaling regulates the decision between immunity and tolerance made by peripheral CD4 (+) T cells. *Int. Immunol.* 2000;**12**:177-185.
27. Vigouroux S, Yvon E, Wagner HJ *et al.* Induction of antigen-specific regulatory T cells following overexpression of a Notch ligand by human B lymphocytes. *J. Virol.* 2003; **77**:10872-10880.
28. Yvon ES, Vigouroux S, Rousseau RF *et al.* Overexpression of the Notch ligand, Jagged-1, induces alloantigen-specific human regulatory T cells. *Blood* 2003;**102**:3815-3821.