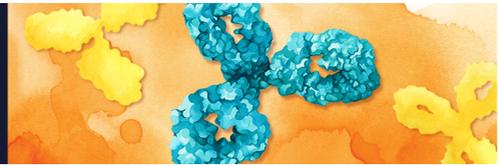


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Th2 Activities Induced During Virgin T Cell Priming in the Absence of IL-4, IL-13, and B Cells¹

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Virgin T cells being primed to Th2-inducing or Th1-inducing Ags, respectively, start to synthesize IL-4 or IFN- γ as they begin to proliferate. Parallel respective induction of B cells to produce $\gamma 1$ or $\gamma 2a$ switch transcripts provides additional evidence of early divergent Th activity. This report concerns the roles of IL-4, IL-13, and B cells in these early events *in vivo*. Th2 responses were induced in lymph nodes against hapten-protein given *s.c.* with killed *Bordetella pertussis* adjuvant. In T cell proliferation in wild-type mice, IL-4 message up-regulation and $\gamma 1$ and ϵ switch transcript production were underway 48–72 h after immunization. The absence of IL-4, IL-13, or B cells did not alter the early T cell proliferative response. The $\gamma 1$ and ϵ switch transcript production was still induced in the absence of IL-4, IL-13, or both, but at a reduced level, while the dominance of switching to IgG1 in the extrafollicular hapten-specific plasma cell response was retained. The up-regulation of IL-4 message was not reduced or delayed in the absence of B cells and was only marginally reduced by the absence of IL-13. It is concluded that signals delivered by dendritic cells, which are not dependent on the presence of IL-4, IL-13, or B cells, can prime virgin T cells and induce the early Th2 activities studied. These early events that direct virgin T cells toward Th2 differentiation contrast with the critical later role of Th2 cytokines in selectively expanding Th2 clones and driving further IL-4 synthesis. *The Journal of Immunology*, 2002, 169: 2900–2906.

The paradigm of bidirectional differentiation of CD4 T cells into Th1 or Th2 effector cells is firmly established (1–4). Equally, it has been repeatedly shown that *in vitro* IL-4 drives the growth of Th2 cells and their secretion of IL-4, while IFN- γ similarly confirms the Th1 phenotype (5). Type 2 cytokines reinforce the Th2 phenotype and expand effector Th2 clones, and uncommitted CD4 T cells induced to proliferate in culture in the presence of IL-4 gradually acquire Th2 characteristics (5–7). Direct cognate interaction between APC and virgin T cells both *in vitro* (8) and *in vivo* (9–11) can induce IL-4 production within 24 h. Therefore, this up-regulation occurs without the T cells undergoing extensive proliferation. These observations point to the possibility that cytokine-independent signals may be able to establish Th2 activity in virgin T cells as they are recruited into immune responses. Analysis of T cell-dependent Ig class switching supports this possibility (11, 12). Thus, when mice are immunized with a mixture of Th1- and Th2-inducing Ags, the Ig class switching induced in B cells specific for each Ag remains remarkably similar to that seen when the Ags are given separately (11). This finding is inconsistent with Th1 and Th2 activity being directed by ambient cytokines in the microenvironment in these responses, although they do not exclude cytokine release at the

immunological synapse between T cells and dendritic cells. T cell-dependent switching to IgG1 is still present, if somewhat reduced, in mice deficient in IL-4 (13, 14). Mice double-deficient in IL-4 and IL-13 have markedly reduced specific IgG1 in recall responses to a protein Ag (15), but as type 2 cytokines expand Th2 clones, analysis of serum Ab titers in secondary responses may disguise conserved switching to IgG1 occurring early in the primary response. The selectivity of switching to IgG1 induced in Th2 responses is emphasized by the switching to IgG2a and IFN- γ production that characterizes the early extrafollicular response to Swiss-type murine mammary tumor virus (11). In this report we test the possibility that cytokine-independent signals are able to direct the development of Th2 effector functions as T cells are primed.

The extrafollicular Ab response provides an opportunity to assess the direction of class switching at an early stage of T cell arming; for the T cell, interaction with B cells is early and transient (12). Thus, during T cell priming B cells can be induced by T cells to up-regulate switch transcript production before the T cells first enter the S phase of the cell cycle (11). Furthermore, B cells proliferate as plasmablasts at distant sites that lack T cells (16, 17); therefore, although T cells direct this growth, they are not present during plasmablast growth. We use this system to test the possibility that *in situ* dendritic cells induce virgin CD4 T cells to show Th2 effector functions in the absence of the major type 2 cytokines, IL-4 and IL-13. As B cells that have taken up Ag interact with T cells from an early stage in priming, the possibility that this liaison influences T cell priming and Th2 development is also probed.

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Materials and Methods

Mice and immunizations

IL-4-deficient mice, IL-13-deficient mice, and IL-4 and IL-13 double-deficient mice, all age and sex matched, have been described in detail previously (14, 15, 18). B cell-deficient MuMT and H chain-deficient ($J_H^{-/-}$) mice have also been described previously (19, 20). The B cell-deficient

phenotype of these mice was confirmed after sacrifice immunohistologically by the lack of IgD-positive, syndecan-1-positive, and IgG subclass-positive cells. Both the genetically deficient, with the exception of the H chain-deficient mice, and wild-type mice were backcrossed with BALB/c for six generations before use in this study.

Alum-precipitated (4-hydroxy-3-nitrophenyl)acetyl (NP)³ conjugated to chicken γ -globulin (CGG) was prepared as previously described (17, 21). Adult mice (8–12 wk) were injected into both rear footpads with 20 μ g alum-precipitated NP-CGG plus 5×10^8 heat-killed *Bordetella pertussis* (Evans Medical, Liverpool, U.K.). Bromodeoxyuridine (BrdU; 2 mg) was administered i.p. 2 h before sacrifice as previously described (16).

Tissue preparation

After death, blood was obtained for the preparation of sera, and draining popliteal lymph nodes were removed. Frozen lymph nodes were sectioned as described previously (11). Five-micrometer thick sections were cut for immunohistology and mounted onto four-spot glass slides. For mRNA extraction, 25- μ m thick sections were cut, placed in polypropylene tubes, and stored at -70°C . Glass-mounted sections were air-dried for 1 h, fixed in acetone (20 min, 4°C), air-dried for 10 min, and sealed in polythene bags at -20°C until use.

NP-specific Ab ELISA

Serum IgG1 and IgG2a Abs to NP were detected by ELISA. NP was conjugated to BSA via a succinimate ester, and this was used to coat an ELISA tray at a concentration of 5 μ g/ml. Sera were adsorbed against goat anti-mouse IgM overnight. Plates were blocked, and primary Ab was added. Each primary Ab was diluted 1/20, then diluted in 5-fold steps three times more. Alkaline phosphatase-linked goat anti-mouse subclass Ab (Southern Biotechnology Associates, Birmingham, AL) was then added. Color was developed using *p*-nitrophenylphosphate in diethanolamine, pH 9.8, as substrate, and plates were read at 405 nm.

Immunohistological reagents, staining, and analysis

Immunohistological reagents and staining were described previously (11, 17). Cells were triple stained for CD3, IgD, and BrdU or double stained for NP and either IgG1 or IgG2a. Rat anti-CD3 (Serotec, Oxford, U.K.) was labeled with biotinylated rabbit anti-rat Abs (Dako, High Wycombe, U.K.), and sheep anti-mouse IgD (The Binding Site, Birmingham, U.K.) was labeled with peroxidase-labeled donkey anti-sheep Ab (The Binding Site). Primary rat anti-mouse Ig Abs (i.e., anti-IgG1 or anti-IgG2a) were labeled using rabbit anti-rat peroxidase Ab (Dako). NP-binding cells were detected using NP-conjugated sheep anti-human IL-2 IgG. Biotinylated rabbit anti-goat Abs (Dako) were used as conjugate to these NP-IgG Abs. After washing, StreptABCComplex-alkaline phosphatase (Dako) was added to sections with biotin-conjugated Abs. HRP was detected using diaminobenzidine tetrahydrochloride solution (17). Alkaline phosphatase activity was detected using naphthol AS-MX phosphate and Fast Blue salt with levamisole (17). BrdU-positive cells were detected as previously described (16). After color development for CD3 and IgD staining, cells were washed and treated for 20 min with 1 M HCl at 60°C . BrdU-containing cells were identified by labeling with a mouse anti-BrdU (Dako) primary Ab and secondarily labeled with a biotinylated goat anti-mouse Ab, and Strept-ABCComplex-alkaline phosphatase (Dako) was added. Color was developed as described above, except that TBS, pH 8.2, and Fast Red TR salt (Sigma) were used. The surface area of lymph nodes was determined using the point-counting technique described by Weible (22).

RT of mRNA and its relative quantification by PCR

Polypropylene tubes containing lymph node sections were removed from the freezer directly onto ice. Cells were lysed directly, and RNA was extracted using RNeasy B (Qiagen, Crawley, U.K.) according to protocol. The RNA pellet was resuspended in 10 mM Tris/0.1 M EDTA buffer (pH 8.0) containing 1 μ g oligo(dT)_{12–18} (Amersham Pharmacia Biotech, Little Chalfont, U.K.) and denatured at 70°C for 10 min. RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Paisley, U.K.) in the presence of 0.01 M DTT, 0.5 mM deoxynucleotide triphosphates, $1 \times$ first-strand buffer, and ~ 20 U RNase inhibitor (Amersham Pharmacia Biotech) at 42°C for 60 min. Reverse transcriptase was inactivated by heating to 90°C for 10 min. Finally, the cDNA was diluted to 100 μ l with 10 mM Tris/0.1 M EDTA buffer, pH 8.0.

Relative quantitation of specific cDNA species to β -actin message was conducted on the ABI 7700 (PE Applied Biosystems, Warrington, U.K.)

using TaqMan chemistry (23) in a multiplex PCR with primers and probes for the target gene and β -actin cDNA in the same reaction vessel. Probes for cytokines and switch transcripts were detected via a 5' label with FAM (PE Applied Biosystems), while probes for β -actin were 5' labeled with VIC (PE Applied Biosystems). Primers and probes were designed using Primer Express according to the manufacturer's directions. Identities of the PCR products were confirmed by DNA sequencing. Sequences were: β -actin forward, CGTGAAAAGATGACCCAGATCA; reverse, TGGTACGAC CAGAGGCATACAG; probe, TCAACACCCAGCCATGTACGTAG CC; γ 1 switch transcript forward, CGAGAAGCCTGAGGAATGTGT; reverse, GGAGTTAGTTTGGCAGCAGAT; probe, TGGCATGGACTAC AGTTGAGAGAACCA; γ 2a switch transcript forward, GGAACAC TAAAGCTGTGACACAT; reverse, AACCTTGACCCAGCCATCTCT; probe, AGCCCCATCGGTCTATCCACTGGC; ϵ switch transcript forward, AAGATGGCTTCGAATAAGAACAGTCT; reverse, CATGGAA GCAGTGCCTTTACAG; probe, CTATCAGGAACCCCTCAGCTCTAC CCTTAAAGC; IL-12 forward, CAAACACCCAGCCATGTACGTAG TT; reverse, TTCTTCTTAATGTCTTCCACTTTTCTT; probe, TCGT GCAGCAAGTGGGCATGTGTT; IFN- γ forward, CATGGCTGTTTC TGGCTGTTAC; reverse, CCAGTTCCTCCAGATATCCAAGA; probe, AACTATTTAACTCAAGTGGCATAGATGTGGAAGAAAAGA; IL-4 forward, GATCATCGGCATTTTGAACGA; reverse, AGGACGTTTG GCACATCCAT; probe, CACAGGAGAAGGGACGCCATGCA; IL-13 forward, TTGAGGAGCTGAGCAACATCAC; reverse, GCGGCCAG GTCCACACT; and probe, CAAGACCAGACTCCCTGTGCAACG. Reaction tubes contained, to a final volume of 25 μ l, $1 \times$ TaqMan Universal PCR Master Mix (PE Applied Biosystems), β -actin-specific primers and probe, target gene-specific primers and probe, and 2 μ l cDNA template. Reaction conditions were the standard conditions for the TaqMan PCR with 60°C annealing temperature, but with 45 PCR cycles. Relative quantification of signal per cell was achieved by setting thresholds within the logarithmic phase of the PCR for β -actin and the target gene and determining the cycle number at which the threshold was reached (C_T). The C_T for the target gene was subtracted from the C_T for β -actin. The relative amount was calculated as $2^{\Delta C_T}$. To obtain the mRNA per lymph node section, the relative amount was multiplied by the section area (in square millimeters).

Statistical analyses

Statistical analysis was conducted using the Mann-Whitney nonparametric sum of ranks test.

Results

Production of IL-4 and cognate interaction with B cells start as T cells are primed

Previous studies show that class-switching to IgG in the response to NP-CGG is almost exclusively to IgG1, even when this Ag is given with an adjuvant, heat-killed *B. pertussis*, which induces switching to IgG2a (11). The first experiments in the present study establish when characteristics of Th2 activity first appear during this response in the draining lymph nodes to primary immunization in the foot. Fig. 1 shows the correlation between the onset of the up-regulation of IL-4 message, the induction of B cells to produce γ 1 switch transcripts, and the time T cells start to proliferate in the T zone. Both γ 1 and IL-4 mRNAs are elevated 48 h after immunization, and by this stage the number of T cells in the S phase of the cell cycle is also significantly increased. By 72 h all three parameters had reached near peak levels. This reflects the histological observation that T cell growth in the T zone is not associated with the accumulation of clusters of activated T cells (16, 17). Thus, T cells produced in the response move apart after dividing, and although some colonize the outer T zone most must either leave the T zone or die in situ. These results provide a baseline to assess the effects of deficiency in type 2 cytokines or B cells in this lymph node response.

B cell and Ig deficiency does not alter the early up-regulation of IL-4 message

The induction of switch transcript production in the response to NP-CGG plus *B. pertussis* is likely to reflect early cognate interaction between B cells and T cells. To test whether this interaction

³ Abbreviations used in this paper: NP, (4-hydroxy-3-nitrophenyl)acetyl; BCR, B cell receptor; BrdU, bromodeoxyuridine; CGG, chicken γ -globulin.

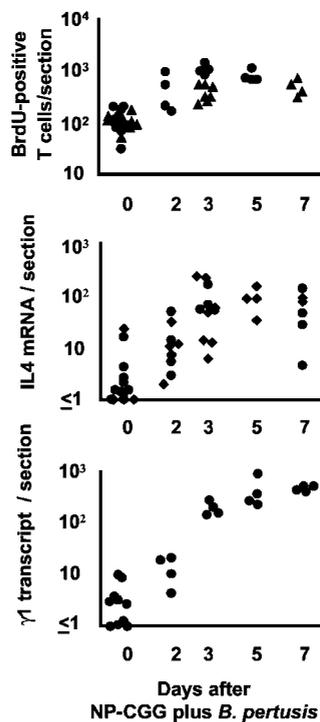


FIGURE 1. Wild-type responses in the draining lymph node to s.c. primary immunization with NP-CGG and *B. pertussis* indicate that by 48 h T cell proliferation and IL-4 up-regulation are underway. By this time cognate interactions between primed T cells and activated B cells have started, with T cells directing B cells to induce $\gamma 1$ switch transcripts. Each symbol type represents a different experiment, and each point represents a single node. mRNA levels are represented relative to β -actin and are adjusted for section size.

is important in inducing up-regulation of type 2 cytokines, the response was studied in μ MT mice, which are deficient in B cells. In these mice IL-4 up-regulation following immunization is not significantly different from that in wild-type congenic controls (Fig. 2A). We are aware of unpublished studies that indicate that some μ MT mice aged 6 mo have detectable IgG in the serum. Consequently we looked at sections of the nodes of μ MT mice for IgD⁺ cells. These were totally absent, but small numbers of B220⁺ cells, up to 50/section, were seen. B220 is also expressed on some T cells (24). The cDNA prepared from these sections contained no detectable $\gamma 1$ switch transcripts (data not shown). In light of the finding of rare B220⁺ cells in μ MT mice an additional experiment was conducted using mice with totally disabled IgH genes (20). These mice also had small numbers of B220⁺ B cells in their lymph nodes, but they had no serum Ig or IgD or IgM⁺ B cells in their lymph nodes (data not shown). They produced an early up-regulation of IL-4 mRNA in their popliteal lymph node response to NP-CGG plus *B. pertussis* (Fig. 2B).

Effects of IL-4 and IL-13 deficiency on the early induction of Th2 activity

Deficiency of either or both these cytokines did not alter the early proliferative T zone T cell response to NP-CGG plus killed *B. pertussis* (Fig. 3A). Although all mice up-regulated $\gamma 1$ switch transcript by day 3 (Fig. 3B), the level was ~ 10 -fold lower in IL-4-deficient mice and was reduced, but to a lesser extent, in mice with IL-13 deficiency. The double-deficient mice showed values similar to those in mice deficient in IL-4 alone. The effect of IL-13 deficiency was almost lost by day 7, but mice deficient in IL-4 still had significantly reduced levels of $\gamma 1$ switch transcript production on

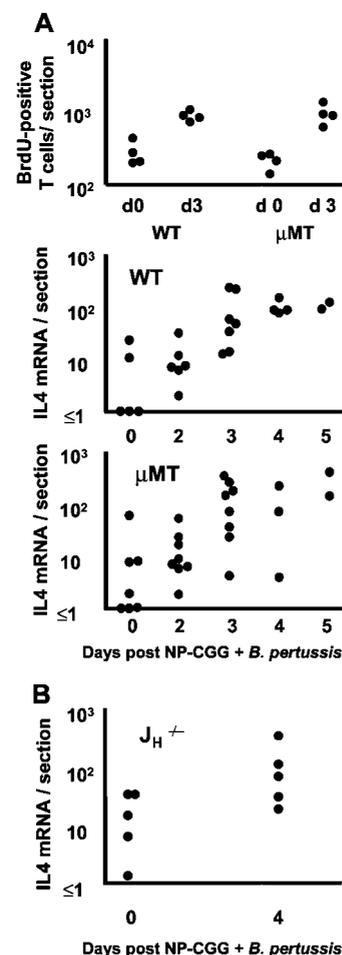


FIGURE 2. B cell-deficient mice and wild-type mice induce T cell proliferation and up-regulate IL-4 mRNA with the same kinetics in the lymph node draining the site of immunization with NP-CGG and *B. pertussis*. Each point represents a single node. Mice were given 2 mg BrdU 2 h before lymph nodes were recovered. Lymph node sections were stained for CD3, IgD, and BrdU. The numbers of CD3 and BrdU double-positive cells in the T zone were counted and adjusted to represent BrdU-positive cells per square millimeter. A, Wild-type mice (WT) and μ MT mice; B, $J_H^{-/-}$ H chain-deficient mice. These mice are on a C57/BL6 background, the littermate controls also show a similar up-regulation, but for clarity these data are not shown.

day 7. There was little effect of IL-13 deficiency on the rate or magnitude of IL-4 mRNA up-regulation. IL-13 mRNA levels did not change significantly in this response in either wild-type or IL-4-deficient mice. An up-regulation of ϵ switch transcript by day 3 was seen in all groups with the exception of the mice deficient in IL-4-alone. By day 7 all groups had significantly more ϵ switch transcripts than on day 3 and before immunization. Nevertheless, as with $\gamma 1$ switch transcript levels, wild-type mice had median ϵ switch transcript levels some 3-fold above those in cytokine-deficient groups (Fig. 3B).

Number of NP- and CGG-specific IgG1 plasma cells produced in the extrafollicular response is relatively conserved in the cytokine-deficient mice

The production of $\gamma 1$ switch transcripts provides an indicator of cognate T cell B cell interaction and the early display of Th2 activity. This does not necessarily reflect the number of switched plasma cells generated. Consequently, total and Ag-specific plasma cell numbers in the medullary cords were enumerated 7

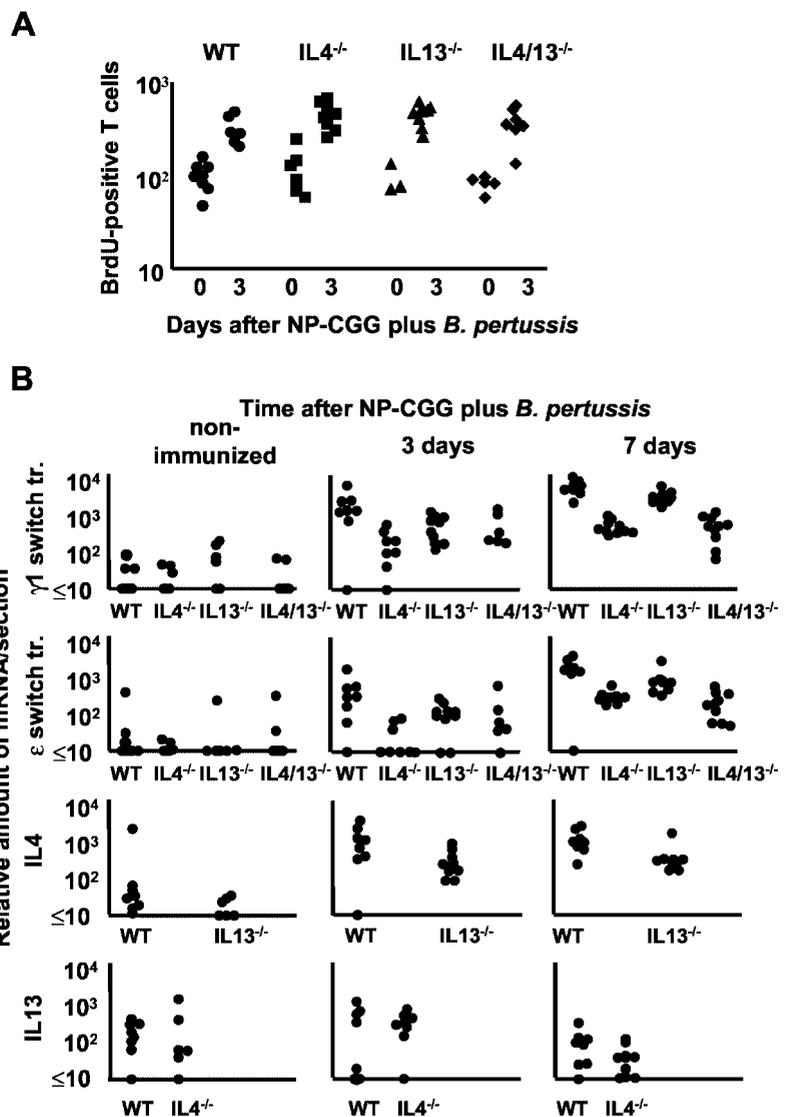


FIGURE 3. Early proliferative and Th2 immune responses in type 2 cytokine-deficient mice immunized with NP-CGG and *B. pertussis* are similar to those seen in wild-type mice. Each point represents a single node. **A**, The numbers of CD3 and BrdU double-positive cells in the T zone were counted and adjusted to represent BrdU-positive cells per square millimeter. **B**, The levels of $\gamma 1$ and ϵ switch transcript and type 2 cytokine message in popliteal lymph nodes before and at intervals after footpad immunization with NP-CGG plus *B. pertussis* in wild-type and type 2 cytokine-deficient mice. In the IL-4 and IL-13 double-deficient mice, no IL-4 transcript was detected using TaqMan primers. In the IL-4-deficient mice, the gene disruption allows the production of a short sterile IL-4 transcript; we have confirmed that there is no full-length IL-4 mRNA production in these mice (data not shown).

days after immunization (Fig. 4). The total numbers of IgG1- and IgG2a-containing plasma cells were comparable in wild-type and type 2 cytokine-deficient mice (Fig. 4B). The proportion of NP-specific plasma cells that had switched to IgG1 was not significantly different between the groups (Fig. 4C). There was a modest, but significant, increase in the proportion of NP-specific cells switching to IgG2a (IL-4^{-/-} vs wild-type, $p < 0.05$; IL-4 and IL-13^{-/-} vs wild-type, $p < 0.001$; Fig. 4D). Similar findings were seen for CGG-specific plasma cells (data not shown). At 7 days after immunization the NP-specific serum IgG1 and IgG2a titers (Fig. 5A) reflect the minor differences seen in plasma cells in the draining node. Interestingly, although single cytokine-deficient mice had comparable germinal center responses 7 days after immunization, mice deficient in both IL-4 and IL-13 had significantly smaller germinal centers ($p < 0.01$; Fig. 5B).

Lack of an early compensatory rise in Th1 activity in the responding nodes in IL-4- and IL-13-deficient mice

An indication that the *B. pertussis* adjuvant had induced Th1 activity in the draining nodes is provided by showing $\gamma 2a$ switch transcripts are produced, and there is an up-regulation of message for IL-12 and IFN- γ . These are not features of responses to alum-precipitated NP-CGG alone (11). By 72 h after immunization (Fig. 6) there is no difference in the $\gamma 2a$ switch transcript levels between

wild-type and cytokine-deficient mice, but after 7 days there is a significant increase in $\gamma 2a$ switch transcript levels in mice deficient in IL-4 (~30-fold compared with day 3, $p < 0.001$), but not in the mice only deficient in IL-13. The IL-12 and IFN- γ levels rose to a small extent in wild-type mice, but paradoxically in response to immunization there was little change in the level of mRNA to these type 1 cytokines in type 2 cytokine-deficient mice.

Discussion

Early events during T cell priming to protein Ags in vivo include the induction of proliferation, the up-regulation of IL-4 mRNA, and cognate interaction with B cells, leading to the production of Ig switch transcripts. In the response studied, all these processes start between 24 and 48 h after immunization and reach near peak levels by 72 h. The T cell B cell interaction is productive, in that activated B cells migrate to the medullary cords where they grow as plasmablasts in the absence of T cells (16), resulting in a well-developed extrafollicular Ab response by day 7. Many of the NP-specific and CGG-specific plasma cells have followed the Th2 switching pattern and produce IgG1. Importantly, this switching is still seen in the absence of the type 2 cytokines IL-4 and IL-13. In addition, despite the early interaction between T and B cells the induction of the T cell proliferative response and IL-4 production was conserved in mice deficient in B cells. It may be argued that

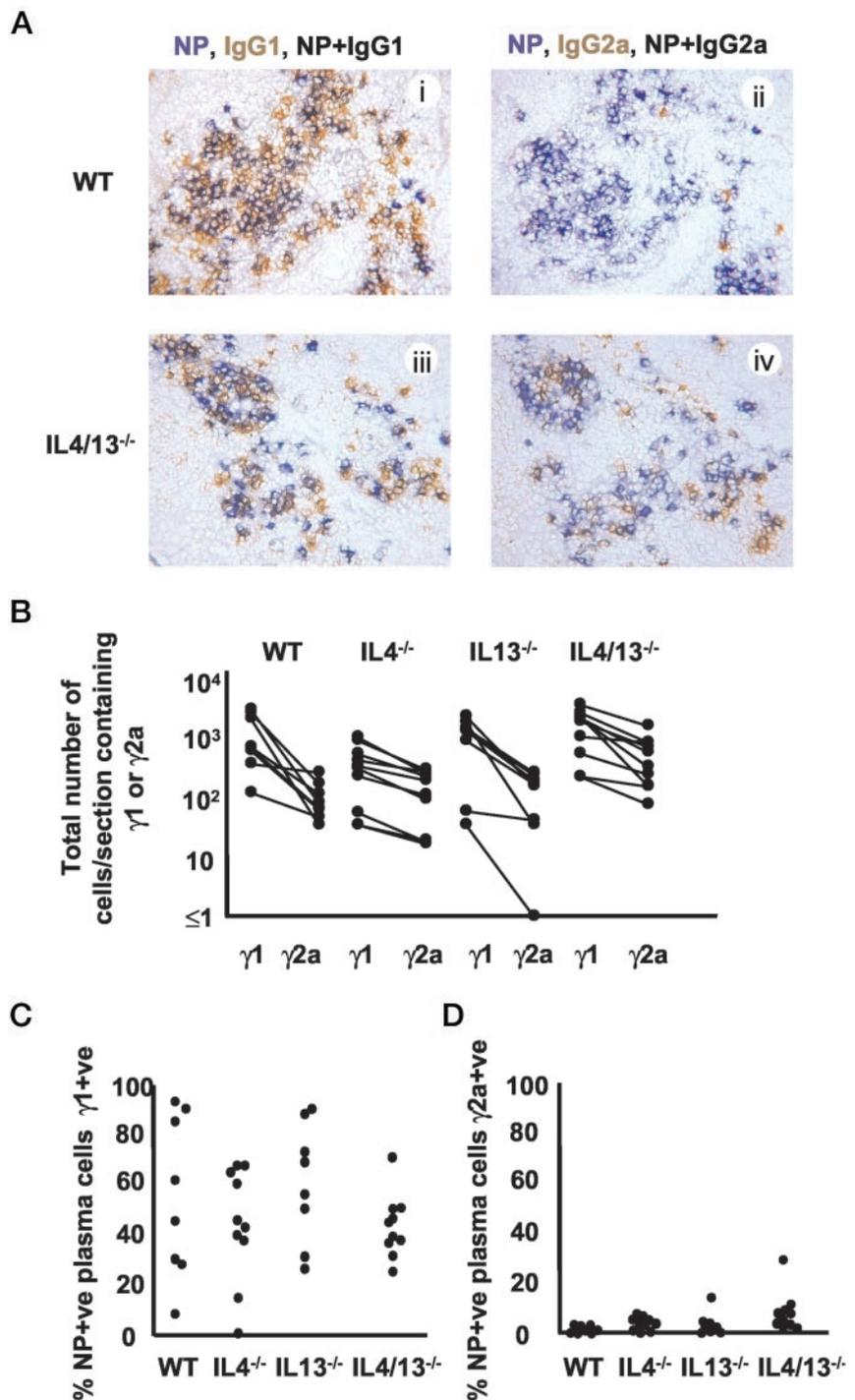


FIGURE 4. Th2 effector T cell-driven differentiation of NP-specific B cells to class switch to IgG1 in the primary extrafollicular response to NP-CGG and *B. pertussis* is virtually unaffected by the deletion of IL-4 and/or IL-13. **A**, Medullary cord plasma cells containing IgG1, IgG2a, and NP-specific Ab 7 days after immunization. NP-specific cells are stained blue; cells containing IgG subclasses are stained brown (IgG1 in *i* and *iii*, IgG2a in *ii* and *iv*); double-stained cells are black. Panels *i* and *ii* are serial sections from congenic wild-type mice, panels *iii* and *iv* are serial sections from IL-4/IL-13 double-deficient mice. **B**, The numbers of IgG1- and IgG2a-expressing plasma cells present in lymph nodes from wild-type and cytokine-deficient mice. The bars link the numbers of IgG1 and IgG2a cells from serial sections from the same node. **C** and **D**, The proportion of NP-specific plasma cells containing IgG1 (**C**) and IgG2a (**D**) in the extrafollicular response 7 days after immunization with NP-CGG and *B. pertussis*. WT, Congenic wild-type. Variations in the numbers of plasma cells in each section are affected by the orientation of the node when sectioning. In some nodes the sections cut through relatively little medulla, and consequently few plasma cells were seen in these sections.

the switching to IgG1 is not a standard characteristic of Th2 activity. Provided this is taken in the context of the strictly Th2-dependent response to NP-CGG, this seems to be a marker of Th2 activity. In this response there is fast up-regulation of IL-4 production, and when *B. pertussis* adjuvant is omitted there is no induction of IFN- γ production (11). The selective induction of IL-4 would generally be accepted to be a Th2 activity. In this response the substantial numbers of NP and CGG-B cells that switch in the extrafollicular response do so almost exclusively to IgG1. Thus in this context the switching to IgG1 is an effect of cells showing Th2 activity. In other contexts, such as in response to NP-Ficolin in T cell-deficient mice, low level switching to IgG1 is patently not Th2 dependent (25). The switching to IgG1 is not

just a generic effect of Th cells, for in the response to Swiss-type murine mammary tumor virus early switching is mainly to IgG2a, and cytokine produced is IFN- γ rather than IL-4 (11).

In recent years major advances have been achieved in determining how Th2 differentiation is driven. T cells appear to exhibit a degree of plasticity in their Th response, as CD4⁺ cells are capable of coproducing IL-4 and IFN- γ mRNA at an early stage during T cell priming (26). When they are cultured for longer in the presence of IL-4 the capacity to synthesize IFN- γ is lost (26, 27). If ambidextrous production of Th1 and Th2 cytokines was a prominent feature of T cells specific for CGG peptides at an early stage in priming, this does not appear to have a major influence in the early induction of Ig class switching. Thus, switching to IgG1 was

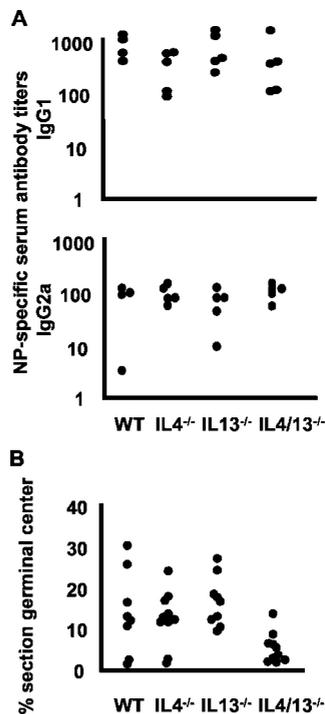


FIGURE 5. NP-specific Ab titers in mice 7 days after immunization with NP-CGG and *B. pertussis*. *A*, Each point represents the value for the serum from a single mouse. Serum titers of nonimmunized mice were <1 in all mice from all four groups. *B*, The areas of germinal centers reflect the combined areas of all germinal centers on each section.

preserved even in the IL-4 and IL-13 double-deficient mice. Even if IFN- γ is produced by CGG-primed T cells in wild-type mice at this early stage it results in only trace numbers of NP- or CGG-specific B cells switching to IgG2a.

There are data that suggest that activated B cells can prime T cells in vitro (28, 29), but this does not appear to be the normal pathway for T cell priming in vivo (30). This is possibly because within secondary lymphoid tissues there is lack of affinity between B cells that have taken up Ag and virgin T cells. B cells do have an important role in expanding and maintaining CD4 T cell memory (31), and the present study confirms (11) that T cells acquire, at a very early stage in priming, the capacity to interact with B cells that have bound Ag and induce these cells to produce switch transcripts. Nevertheless, this interaction is shown not to be necessary for the early induction of IL-4 production. It has been reported that T cells primed in the absence of B cells may lack the capacity subsequently to direct Ag-presenting B cells to switch to IgG (32). This was not tested in the present study. NK1.1 T cells can produce IL-4, but early IL-4 production in lymph node responses have found that this is attributable to CD4⁺ T cells other than NK1.1 cells (33, 34).

There have been other reports that early Th2 differentiation events can be triggered in the absence of IL-4 signaling. Lymphocytes producing IL-4 and IL-5 were induced by helminth infections in mice deficient in either IL-4R or its downstream target, STAT-6 (35, 36). This suggests that IL-4 is just one means to activate a pathway that drives Th2 differentiation. Our studies suggest that this alternative IL-4-independent pathway plays a central role in directing primary Th2 commitment in vivo. Supporting evidence for this comes from recent investigations of the role of the transcription factor STAT-6 after activation in vitro with IL-4 (37). STAT-6 itself has a regulatory role on two other transcription factors c-Maf and GATA-3, which appear to have critical roles in

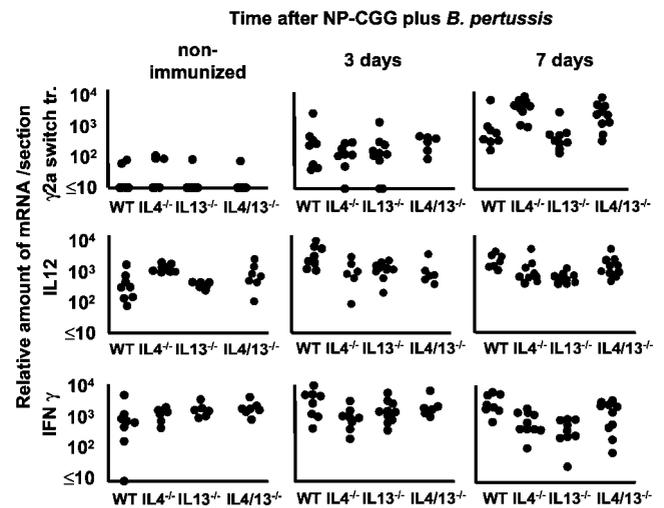


FIGURE 6. In the early primary immune response to NP-CGG and *B. pertussis* there is minimal primary immune response to NP-CGG and *B. pertussis* there is minimal primary immune response of markers of Th1 activity in mice deficient in IL-4 and/or IL-13. The levels of $\gamma 2a$ switch transcript are only elevated above wild-type 7 days after primary immunization. Type 1 cytokine message is, if anything, up-regulated to a lesser degree than in wild-type mice.

the induction of Th2 responses (38); c-Maf has an important role in up-regulating IL-4, and GATA-3 can drive Th2 responses in a STAT-6- and IL-4-independent fashion. GATA-3 also seems to be able to redirect clones from a Th1 to a Th2 phenotype (26, 39–43). Thus, the evolutionary importance in maintaining Th2 activity has led to the ability to initiate Th2 responses through multiple pathways. Nevertheless, there is still an important role for IL-4 in the emergence of Th2 clones in vivo. This cytokine has repeatedly been shown to be essential for reinforcing and maintaining the Th2 phenotype and expanding their numbers (13, 14).

The present study confirms our previous observation that cells showing Th1 activity can emerge at the same time in the same lymph node as cells with a Th2 phenotype (11). The early onset of this divergent differentiation and its independence from B cells point to the instruction for differentiation being delivered by T cell interaction with a dendritic cell. This, in turn, is likely to reflect the way the dendritic cell is induced to take up Ag and the nature of the Ag. Recent progress in dendritic cell biology has begun to unravel how these cells may regulate T cell differentiation. It has been reported that in vivo different subsets of dendritic cells are able to prime distinct Th responses, but whether this is an intrinsic property of these cells or is contextual depending upon the cell's environment is not entirely clear (44–47). An alternative, but not mutually exclusive, explanation is that a common dendritic cell precursor processes different Ags in different ways. Thus, the stage in endosomal maturation when peptides associate with class II MHC molecules may reflect the mix and amount of costimulatory molecules that are brought from the endosome to the cell surface. These questions are starting to be resolved by multiparameter microscopic studies of ever increasing resolution of cognate encounters between virgin T cells and dendritic cell in vivo (48).

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