

B Cells Solicit Their Own Help from T Cells

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Summary

We have made use of T cell receptor (TCR)–transgenic mice with CD4⁺ T cells expressing a receptor specific for the self-antigen C5 (fifth component of complement) to study the role of different antigen-presenting cells in the determination of CD4⁺ T cell effector type. Contact of T cells from C5 TCR–transgenic mice with C5 protein or C5 peptide *in vivo* or *in vitro* induces biased T helper cell (Th) 1 type responses resulting in exclusive production of high levels of interferon γ and interleukin (IL) 2. Transgenic mice, in contrast to nontransgenic littermates, do not generate an antibody response to C5. We show in this paper that B cell presentation *in vitro* induces a switch to the Th2 subset indicated by production of IL-4, and targeting C5 to B cells *in vivo* results in the generation of C5-specific antibodies.

Activation of CD4⁺ T cells leads to their differentiation into subsets that are characterized by their function and lymphokine secretion pattern. Th1 cells secrete IL-2, IFN- γ , and TNF- β , which are involved in macrophage activation and induction of delayed-type hypersensitivity responses. The lymphokines typical for Th2 cells are IL-4, IL-5, and IL-10 and are critical for IgG1 and IgE antibody production (1). These two T cell subpopulations regulate each others' function through the antagonistic activity of their respective cytokines (2, 3). Th lineage commitment is of crucial importance with respect to susceptibility or resistance to particular infections or autoimmune diseases. A number of factors determine whether an immune response will be dominated by Th1 or Th2 cells: cytokines acting in the microenvironment, and to some extent contributed by the innate immune system, play an important role in lineage differentiation. IL-12 secretion by macrophages and dendritic cells drives differentiation of Th1 effector cells (4–6), and IL-4, which can be T cell derived or secreted by CD4⁺ NK-like cells or basophils, drives Th2 development (7–13).

However, the cytokine environment is not the only parameter to regulate the differentiation of CD4 T cells. A host of recently published data has implicated differential regulation through costimulatory molecules (14, 15), the effect of ligand density (16), and variations in signaling (17, 18) as instrumental in driving the commitment of CD4 T cells to either Th1 or Th2.

The use of TCR transgenic mice has made it possible to investigate the switching of Th phenotype within a monoclonal T cell population and has provided compelling evidence for the crucial role of IL-12 in Th1 differentiation (3). We have made use of another TCR transgenic mouse

system with CD4⁺ T cells expressing a receptor specific for the self-antigen C5¹ (the fifth component of mouse complement) (19). Contact of T cells from C5 TCR–transgenic mice with C5 protein or C5 peptide *in vivo* or *in vitro* induces biased Th1-type responses resulting in exclusive production of IL-2 and high levels of IFN- γ . In contrast to nontransgenic littermates, which generate C5-specific antibodies, C5 TCR–transgenic mice do not develop an antibody response to C5. We used these transgenic mice to investigate the role of APC in the determination of CD4⁺ T cell effector type.

In this article we show that T cell help for B cells and the induction of IL-4 production are induced by activated B cells presenting C5. Under physiological conditions *in vivo*, C5 may be preferentially presented by dendritic cells and macrophages, resulting in skewing to the Th1 pathway. Increasing B cell presentation *in vitro* and targeting C5 for B cell presentation in adoptive transfer experiments *in vivo* induces the production of IL-4 and generation of C5-specific antibodies.

Materials and Methods

Animals. A/J (C5⁻) mice are maintained under specified pathogen-free conditions at the National Institute for Medical Research, Mill Hill. The congenic strain A.C5⁺ was kindly provided by Dr. F. Gervais (Montreal General Hospital Research Institute, Montreal, Canada) (20) and is now bred in Mill Hill. The generation of TCR–transgenic mice carrying a TCR specific for C5 was described in detail (19).

¹Abbreviations used in this paper: C5, fifth component of complement; MACS, magnetic cell sorting.

Antibodies. The following antibodies were used in the experiments: anti-IL-4 (11B11 ATCC HB 188), anti-IL-2 (S4B6 [21]), anti-CD8-FITC labeled, anti-CD4-PE labeled (Boehringer Mannheim Corp., Indianapolis, IN), anti-IFN- γ , R46A2 (22), and AN-18 (23). Two antibodies to mouse CD40 were used in this study: FGK-45, a rat IgG/ κ made and generously provided by Drs. J. Andersson and A. Rolink (Basel Institute for Immunology, Basel, Switzerland), and DOM1-85, a rat IgG/ κ made in our own laboratory (D. Gray). Both antibodies were raised by immunizing rats with alum-precipitated CD40-human IgG1 soluble fusion protein. Positive clones reacted with the fusion protein but not human IgG1 in ELISA; the antibodies have the expected staining patterns on cell suspensions on the FACS[®], stimulate B cells to undergo homotypic adhesion and proliferation, do not bind to cells from CD40 knockout mice, and detect a 50-kD band in Western blots of B cell lysates.

APC. LK35 lymphoma/hybridoma cells (ATCC 98; American Type Culture Collection [ATCC], Rockville, MD) were used as a source of B cells. For optimal expression of B7 molecules, LK35 were cultured in the presence of 20 μ g/ml LPS (*Escherichia coli*; L-2880; Sigma Chemical Co., St. Louis, MO) for 24 h before functional set ups. 97.2, a macrophage line derived from bone marrow of AKR mice, was a gift from Dr. R. Palacios (M.D. Anderson Cancer Center, Houston, TX). Macrophages were activated for MHC class II expression by culture with 200 U/ml recombinant IFN- γ (derived from a cell line transfected with IFN- γ cDNA (24) 48 h before functional tests. Dendritic cells were derived from bone marrow cultures in the presence of GM-CSF as described in detail (25). The source of GM-CSF was supernatant from HAT-sensitive Ag8653 myeloma cells transfected with murine GM-CSF cDNA, which was isolated from a T cell clone by PCR and inserted into the vector BCMGSNeo, kindly provided by Dr. H. Karasuyama (Basel Institute for Immunology) (24).

Functional T Cell Activation Tests. B cells and macrophages, pretreated as described above, were treated with mitomycin C (50 μ g/ml; M-0503; Sigma Chemical Co.), plated into 96-well round-bottom tissue culture plates (at 5×10^4 cells/well or 10^3 cells/well for dendritic cells) and incubated in the presence of C5 peptide 106-121 in serum-free medium for 3 h at 37°C. Peptide was washed out, and 5×10^5 cells/well of transgenic thymocytes were added as a source of naive T cells. The culture medium was IMDM supplemented with 5% heat-inactivated FCS, 2×10^{-3} M L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 5×10^{-5} M ME. Supernatant from cultures incubated for 48 h was tested for the presence of IL-2 or IL-4, respectively, by its ability to support the growth of the IL-2-dependent cell line CTLL (ATCC TIB 214) or the IL-4-dependent cell line CT4S (26). Residual activity of CT4S cells against IL-2 was blocked by addition of anti-IL-2 antibody S4B6. Supernatant from cultures incubated for 72 h was tested for IFN- γ activity by a sandwich ELISA as previously described (27). Briefly, antibodies to mouse IFN- γ were used for coating of ELISA plates, followed by addition of supernatants to be tested for the presence of IFN- γ , capture by a second biotinylated IFN- γ -specific antibody, and detection with streptavidin conjugated with horseradish peroxidase.

Immunization and Determination of Antibody Titers. Mice were immunized with 10 μ g alum-precipitated protein subcutaneously above the footpads. For determination of antibody titers, microtiter ELISA plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with the test antigen at 10 μ g/ml; 50 μ l/well in carbonate/bicarbonate buffer, pH 9.5. Antibody titers were worked out by setting up doubling dilutions of serum, starting at 1 in 20. Curves of absorbance (at 405 nm) against serum dilution were

plotted. The antibody titer was calculated as the reciprocal of the dilution that gave an absorbance of 30% of the maximum absorbance reading for that particular assay. The maximum was given by a standard hyperimmune serum. Secondary antiisotype antibodies used in these assays were the following: alkaline phosphatase conjugates of goat anti-mouse IgG1, goat anti-mouse IgG2a, and goat anti-mouse IgM (all purchased from Southern Biotechnology Associates, Birmingham, AL).

Sorting of Thymocytes. Transgenic thymocytes were double labeled with anti-CD8-FITC and anti-CD4-PE (Boehringer Mannheim Corp.) and sorted for single CD4⁺ cells and double-positive cells using a FacsStar[®]Plus (Becton Dickinson & Co., Mountain View, CA) equipped with a laser working at 488 nm.

Magnetic Cell Sorting (MACS) Preparation of B Cells. Positive selection of B cells from spleens of CBA nude mice was done by MACS with the Vario-MACS (Miltenyi Biotech, Bergisch Gladbach, Germany) using goat anti-rat Ig beads and following the procedure recommended in the manual. Positively selected cells were passed over the selection column twice, and the purity of the selected population was between 80 and 90%.

Results

C5 TCR-transgenic Mice Generate Biased Th1 Responses to C5. Spleen cells from C5 TCR-transgenic mice on a C5⁻ (A/J) background stimulated in vitro with either C5 protein or C5 peptide generate strong IL-2 and IFN- γ responses. Spleen cells from nontransgenic A/J mice do not make primary in vitro responses to C5 (Fig. 1 A). Immunization with C5 protein resulted in C5-specific IgG antibody production in nontransgenic mice but not in transgenic mice, which make IgM antibodies, but no switched isotypes (Fig. 1 B). The isotype of C5-specific antibodies in normal mice was exclusively IgG1: there was no IgG2a produced and very little IgG2b (not shown). This phenotype was observed regardless of the mode of immunization; that is, C5 precipitated with alum injected with or without pertussis toxin intraperitoneally or subcutaneously, or C5 in CFA injected subcutaneously, resulted in IgG1 antibody formation in nontransgenic but not transgenic C5⁻ mice. This indicates that contact with C5 in vivo and in vitro induces a preferential Th1 response in the transgenic mice. Cytokine effects due to differences in the genetic background are not likely to play a role in preferential differentiation to the Th1 effector class since nontransgenic littermates readily made C5-specific antibodies of the Th2-dependent IgG1 isotype. We therefore decided to investigate the role of different APC in influencing effector class development.

Naive Resting Transgenic T Cells Develop into Th1 Effector Cells upon Contact with Antigen Presented by Dendritic Cells. As a source of naive transgenic T cells, we used thymocytes. Functional activity resides exclusively in the CD4 single-positive subpopulation, which constitutes ~8% of total thymocytes. Fig. 2 shows that sorted CD4 single-positive thymocytes generated IL-2 and IFN- γ in response to C5 presented by dendritic cells. No IL-4 production was detectable. The sorted double-positive thymocyte population was not activatable at any cell dose used. For the ex-

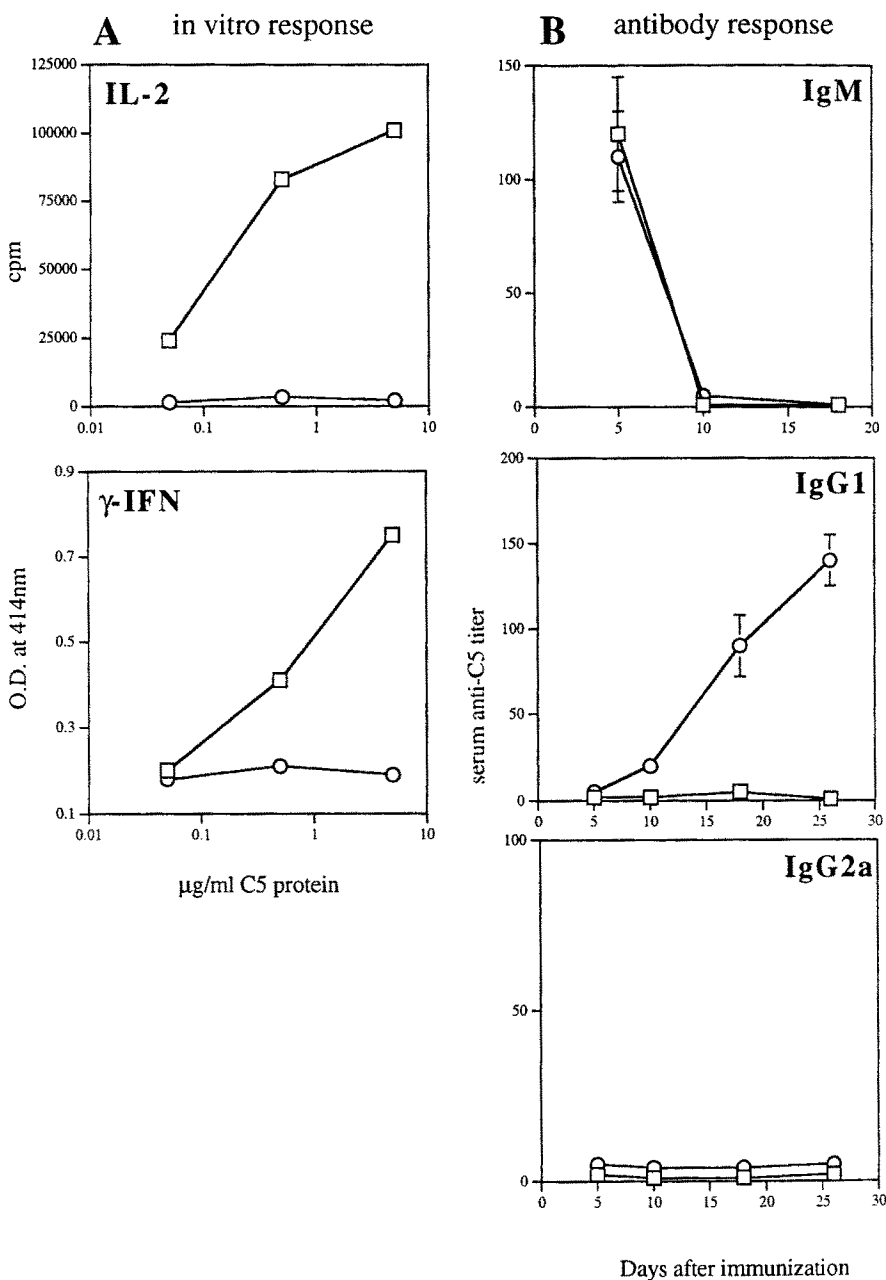


Figure 1. (A) Spleen cells from A18.A transgenic mice (squares) or nontransgenic A/J mice (circles) were cultured at 2×10^5 cells/well with C5 protein. 48 h later they were assessed for production of IL-2 by transfer of supernatant to the IL-2-dependent CTLL line and 72 h later for production of IFN- γ by ELISA. The upper panel shows [3 H]thymidine incorporated into IL-2-dependent CTLL cells; the lower panel shows arbitrary values of OD at 414 nm as assessment of IFN- γ bound to ELISA plates. (B) A18.A transgenic mice (squares) or nontransgenic A/J mice (circles) were immunized with 10 μ g alum-precipitated C5 protein subcutaneously. At different time points after immunization, mice were bled and assessed for production of C5-specific antibodies of IgM, IgG1, and IgG2a subclass by ELISA. The graph shows antibody titers of four mice per group.

periments shown from now on, total unsorted thymocytes were used as a source of naive T cells.

Cytokine Profiles Are Influenced by Different APC. To test the effect of different APC on cytokine profiles, we used APC cell lines to avoid problems with purification and the inevitability of contamination. LK35 lymphoma/hybridoma cells activated by LPS for optimal expression of costimulatory molecules were used as a source of activated B cells. In addition, we used the macrophage line 97.2 activated by IFN- γ and dendritic cells generated from bone marrow cultures in the presence of GM-CSF. APC pulsed with C5 peptide in a range from 10 μ M to 10 nM were cultured with transgenic thymocytes for either 48 h (optimal release of IL-2 and IL-4) or 72 h (optimal release of IFN- γ). Fig. 3

A shows IL-2, IL-4, and IFN- γ release by thymocytes cultured in the presence of either B cells, macrophages, or a very low number of dendritic cells. B cells and macrophages alone stimulated low IL-2 responses, but no IFN- γ or IL-4 response. 10^3 dendritic cells on their own activated naive T cells for IL-2 and IFN- γ production. The IFN- γ responses were variable and often much higher than in this experiment, but no IL-4 responses were observed in any experiment. Since dendritic cells are essential to initiate optimal responses from naive T cells, we supplemented B cells and macrophages with 10^3 (2% of total APC) of dendritic cells. Macrophages supplemented with dendritic cells stimulated strong IL-2 and IFN- γ responses, but no IL-4 production was detectable (Fig. 3 B). In contrast, B cells supplemented with

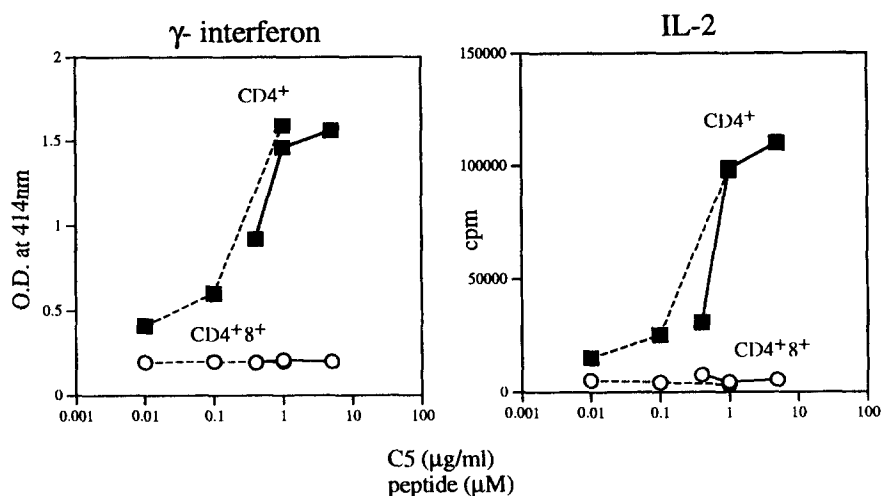


Figure 2. Thymocytes from A18.A transgenic mice were sorted by FACStar[®] for CD4 single-positive cells (*squares*) or CD4⁺8⁺ double-positive cells (*circles*). 10⁵ sorted cells/well were cultured in the presence of 2 × 10⁴ dendritic cells (from bone marrow cultures with GM-CSF) and various doses of either C5 protein (*solid lines*) or C5 peptide (*dashed lines*). After 48 h, supernatants were assessed for the presence of IL-2, and after 72 h, for the presence of IFN- γ .

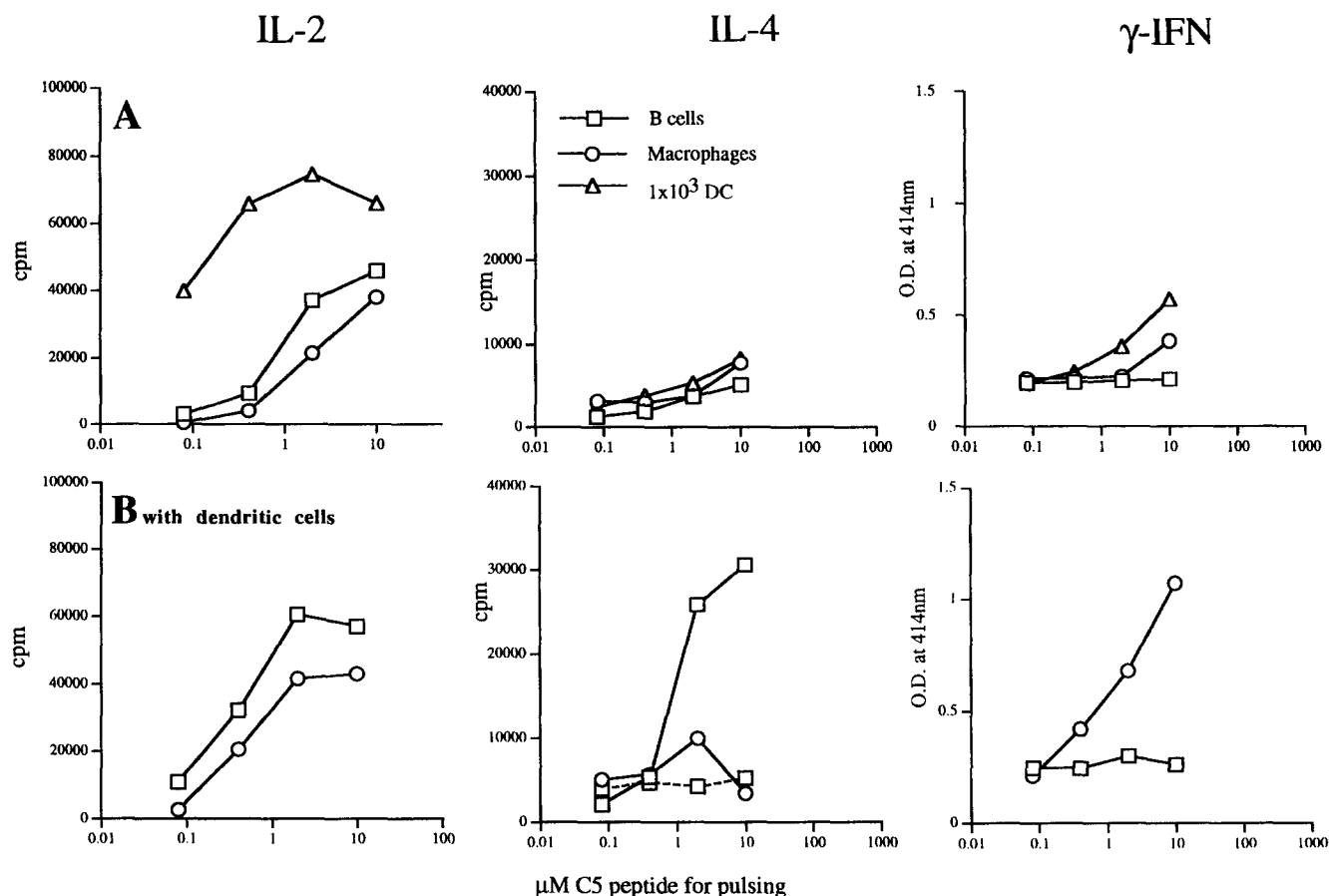


Figure 3. (A) 5 × 10⁵ unseparated thymocytes per well from A18.A transgenic mice were cultured for 48 h (IL-2 and IL-4 assessment) or 72 h (IFN- γ assessment) in the presence of different peptide-pulsed APC. These were either LK35 B cells (*squares*; 5 × 10⁴ cells/well) pretreated with LPS (20 μ g/ml) for 24 h before the assay, 97.2 macrophages (*circles*; 5 × 10⁴ cells/well) pretreated for 48 h before the assay with 200 U/ml recombinant IFN- γ , or dendritic cells (*triangles*; 10³ cells/well) from bone marrow cultures. B cells and macrophages were treated with mitomycin C, and all APC were pulsed with the indicated doses of peptide in serum-free medium for 3 h at 37°C. Peptide was washed out of the cultures extensively before addition of T cells. IL-2 production was assessed by proliferation of IL-2-dependent CTLL cells, IL-4 production was assessed on IL-4-dependent CT4S cells in the presence of anti-IL-2 antibody, and IFN- γ was assessed by ELISA. (B) B cells and macrophages were mixed with 2% dendritic cells (10³/well), and the experimental set up was otherwise identical to that described in A. The squares with dashed lines in the IL-4 panel represent the IL-4 response induced by B cells plus 2% dendritic cells in the presence of anti-IL-4 antibody (10 μ g/ml 11B11).

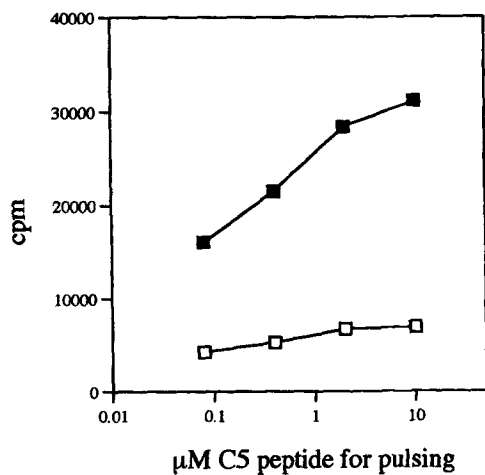


Figure 4. IL-4 production induced by normal B cells. 5×10^5 unseparated thymocytes per well from A18.A transgenic mice were cultured with 5×10^4 B cells per well positively selected from spleens of CBA nude mice by MACS. Open symbols represent the IL-4 response obtained after culture with freshly isolated B cells, and solid symbols represent the response induced by B cells activated for 24 h by LPS ($20 \mu\text{g/ml}$).

dendritic cells stimulated not only release of IL-2, but also IL-4. The IL-4 release was abrogated in the presence of anti-IL-4 antibody. Interestingly, the IFN- γ responses normally detected with dendritic cells on their own were suppressed in the presence of B cells. IL-4 responses in the presence of B cells were detected in four out of six experiments. In the two experiments that did not show IL-4 release by transgenic T cells, IFN- γ was detected instead, indicating a delicate balance in the switch to either Th1- or Th2-type cytokines. These results show that APC influence the cytokine profile of CD4 T cells and indicate that B cell presentation is essential for a switch to IL-4 production.

Presentation by Normal B Cells Induces Th2 Responses. The use of cell lines as APC was justified to avoid problems of cross-contamination and heterogeneity of APC preparations. However, we were concerned about the possibility that the B cell tumor line might itself produce Th2-inducing factors

and therefore not resemble normal B cells. Therefore we repeated the experiments with B cells purified from mice by positive selection with MACS beads. Freshly isolated B cells pulsed with peptide *in vitro* did not induce IL-4-producing T cells, but B cells activated by LPS for 24 h initiated a strong IL-4 response comparable to that seen after stimulation with the B cell line (Fig. 4).

Anti-CD40 Antibodies Suppress Generation of IL-4 Responses. To investigate which cell surface molecules are involved in the interaction between B cells and T cells to promote a switch to IL-4, antibody-blocking studies with antibodies to CD4, B7, and CD40 were carried out. Whereas anti-CD4 and anti-B7 antibodies decreased or abrogated all cytokine responses (data not shown), anti-CD40 had a very discriminatory effect. As shown in Fig. 5, two different anti-CD40 antibodies suppressed the production of IL-4 in the presence of B cells, and instead provoked an IFN- γ response. We conclude that an interaction between CD40 expressed on B cells and CD40 ligand on T cells is necessary to signal a switch to IL-4 production.

B Cell Presentation *In Vivo* Is Critical for the Development of Th Cells. Given that B cells appear to play a crucial role in determining the developmental pathway of CD4 T cells, as indicated by cytokine release *in vitro*, a crucial question was whether they play this role *in vivo*. Since our transgenic mice do not develop antibodies to C5, the underlying assumption is that presentation by macrophages and dendritic cells overrides B cell presentation and skews effector cell development into the Th1 lineage. To target antigen preferentially to B cells, we used a hapten carrier adoptive transfer system. For this, we used DNP-specific B cells that internalized haptened DNP-C5 and subsequently processed C5 peptides for presentation to transgenic T cells. Two groups of transgenic mice were immunized as shown in the scheme in Fig. 6. Group I received 10^6 bone marrow-derived dendritic cells that had been pulsed with C5 protein and were coinjected with soluble haptened DNP-C5. Group II received 10×10^6 DNP-immune B cells purified from C5⁺ mice immunized with DNP on ovalbumin as carrier protein. It should be pointed out that these donor mice, which

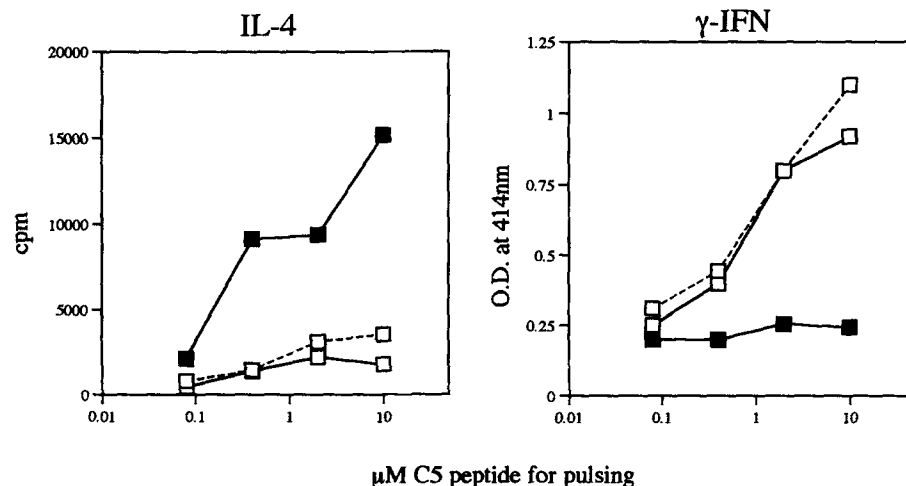


Figure 5. 5×10^5 unseparated thymocytes per well from A18.A transgenic mice were cultured with a mixture of mitomycin-treated LK35 cells (5×10^4 /well) and dendritic cells (10^3 /well) that were pulsed together with the peptide doses indicated on the abscissa, in the same manner as described in the legend to Fig. 3. IL-4 and IFN- γ production were assessed in the absence (solid squares, solid lines) or presence of $20 \mu\text{g/ml}$ anti-CD40 antibodies. (Open squares, solid lines anti-CD40 antibody FGK-45, and open squares, dashed lines anti-CD40 antibody DOM1-85). IL-4 production was assessed after 48 h of culture, and IFN- γ was assessed after 72 h.

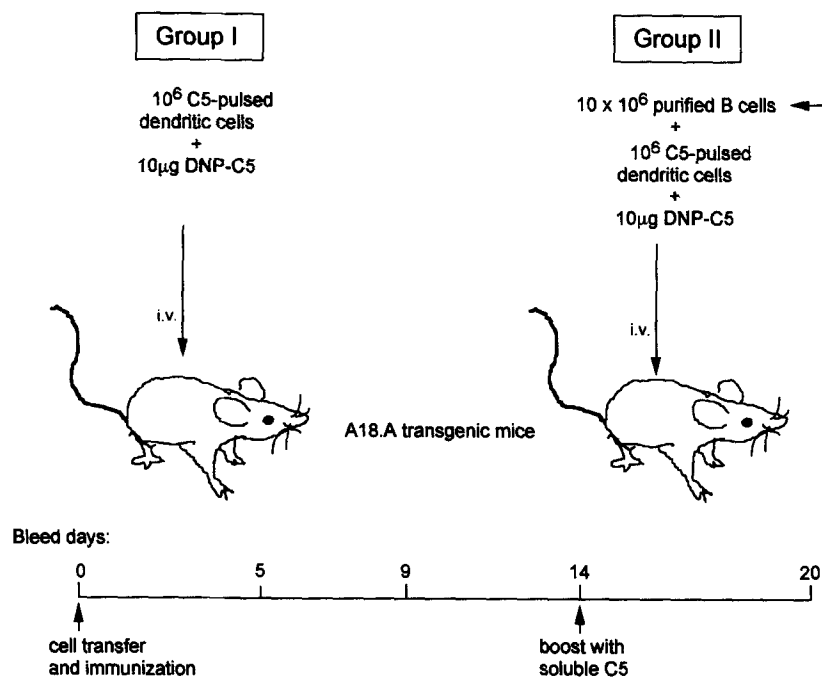


Figure 6. Immunization scheme. Priming of transgenic T cells in the presence of specific B cells allows anti-C5 antibody responses.

have C5 in their circulation, contain neither C5-specific T cells nor C5-specific B cells since they are tolerant of their own C5. In addition to the B cells, 10^6 C5-pulsed dendritic cells as in Group I were included to optimize activation of naive T cells in the adoptive host: thus, Group II mice received B cells, dendritic cells, and soluble DNP-C5. At various times after injection, the mice were bled and tested for IgG1 and IgG2a antibody production against DNP-BSA or C5. Some mice were boosted with soluble unconjugated C5 at day 14 after transfer. The results shown in Fig. 7 provide evidence that antigen targeting to B cells allows the development of helper T cells supporting an antibody response. Group II mice made antibodies against DNP of IgG1 and IgG2a subclass, which were presumably derived from the donor inoculum of DNP-immune B cells. It should be stressed that DNP immune B cells are dependent on T cell help for reactivation to antibody production. This was verified by injecting them into C5^+ mice together with DNP-C5, where they failed to make anti-DNP antibodies because of the absence of carrier-specific helper T cells (data not shown). More importantly, in addition to DNP antibodies, Group II mice generated C5-specific antibodies, which could only have been derived from the endogenous B cell population in the transgenic host. Boosting with soluble C5 further increased this response. In contrast, none of the Group I mice made any antibody responses despite the fact that injection with antigen-pulsed dendritic cells results in strong T cell activation (data not shown). Interestingly, C5-specific antibodies detected in Group II transgenic mice consisted of both IgG1 as well as IgG2, whereas in normal mice the IgG1 response predominates and IgG2a is never detected (see Fig. 1 B).

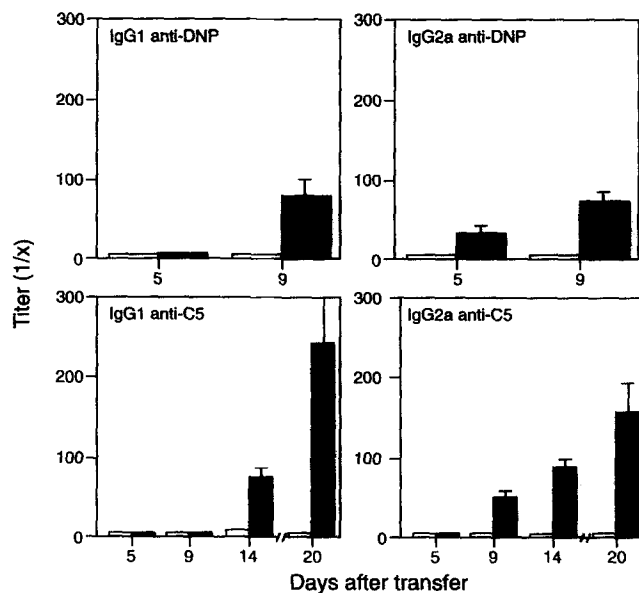


Figure 7. Two groups of A18.A transgenic mice (four mice per group) were injected as illustrated in the scheme in Fig. 5. One group received 10^6 dendritic cells that had been pulsed with $10\mu\text{g/ml}$ C5 protein for 6 h before injection, washed, and coinjected intravenously with $10\mu\text{g}$ DNP-C5 in PBS. The second group received a mixture of C5-pulsed dendritic cells and 10×10^6 MACS-purified B cells from congenic A.C5^+ donors primed 3 wk previously with DNP-ovalbumin. The cells were coinjected intravenously with $10\mu\text{g}$ soluble DNP-C5. At different time points after injection, the mice were bled and assessed for IgG1 and IgG2a antibodies specific for DNP or C5. Antibody titers of four mice per group are shown. □, Group I (DC only); ■, Group II (DC + B).

Discussion

It was shown several years ago that the basis of cognate T cell help for B cells resides in antigen presentation by B cells and the subsequent display of class II-bound peptides recognized by specific T cells so that cytokine release is targeted to antigen-specific B cells (28). In this article we show that the influence of B cell presentation to T cells is even more pervasive as it directly influences the differentiation of T cells into Th2 helper cells.

A number of studies have analyzed stimulation of different CD4 effector cells by various APC (29, 30), or the role of APC in initiation of Th1 or Th2 development from naive T cells (7, 8, 31–33). With the exception of the clearcut influence of macrophages on Th1 differentiation, there was no obvious correlation between a particular type of APC and the development of either effector class, and a candidate APC for promotion of Th2 responses has not been identified so far. What seems clear is that under certain cytokine conditions, that is, in the presence of IL-4, any APC will stimulate a Th2 response (7, 31, 34). The crucial point of our study is that in the absence of a favorable cytokine environment, presentation of antigen by B cells can override stimuli that otherwise will skew development to Th1 phenotype. What are these stimuli? It has been pointed out that the ligand density available for T cell recognition plays an important role, with high ligand density favoring differentiation of Th1 cells and low ligand density favoring differentiation of Th2 cells (16). It seems likely that the effective ligand density on different APC varies, depending on the way they take up antigen. Thus, ligand density may depend on the nature of the antigen and which APC preferentially or initially presents it. Although there is no information available about the actual ligand densities created for complex proteins like C5 on different APC, we do have evidence that it is handled quite differently by different APC (35).

C5 is very efficiently presented by dendritic cells and also by macrophages, both of which have been shown to produce IL-12, which drives development of Th1 cells (4, 5). In fact, we know that under physiological conditions, dendritic cells presenting C5 are the only APC that can activate resting T cells (36). A caveat is that we have no information about antigen-specific B cells, which in other systems have been shown to be capable of activating resting T cells (37). Our *in vitro* data show that B cell presentation can result in a switch to Th2 mode. The B cells used for these experiments were artificially turned into antigen-specific B cells expressing costimulatory molecules and high amounts of peptide on class II. Whether this accurately reflects truly antigen-specific B cells *in vivo* is debatable. However, it was evident that switching to Th2 responses was achieved whether the source of B cells was a tumor cell line or normal B cells isolated directly from mice. The *in vitro* studies suggest that some dendritic cells had to be present to initiate a T cell response in the first place. However, when dendritic cells are overrepresented, a switch to Th1 development was inevitable.

A limited analysis of the molecular basis of this Th2-inducing activity of B cells revealed that blocking the CD40–CD40 ligand interaction with anti-CD40 antibodies inhibited the release of IL-4, but not IL-2 and IFN- γ , whereas B7 blockade impaired all cytokine release. The interpretation we favor is that costimulatory signals to T cells delivered via CD40 ligand are responsible for Th2 induction. This is in line with the observation that T cells primed in CD40 knockout mice do not develop full helper function; they do not support IgG responses of normal (CD40⁺) B cells (37).

This brings up an issue that at first sight may seem paradoxical. Why is the interaction of CD40 on B cells with CD40 ligand on T cells involved in a switch to Th2 when dendritic cells constitutively express CD40 themselves? The answer to this question must be twofold. First, at the time when the first interaction of a dendritic cell with a naive T cell occurs, this T cell does not yet express CD40 ligand. The capacity of dendritic cells to initially activate a resting T cell is therefore independent of a CD40 interaction. Once the T cell does express CD40 ligand, subsequent interactions involving CD40 on dendritic cells will nevertheless not induce a Th2 switch, but rather the opposite, since dendritic cells are potent producers of IL-12 (4). Although IL-12 cDNA was originally isolated from EBV-transformed B cells (38), and murine B cell lines are reported to produce IL-12 (39), the conditions under which normal B cells secrete this cytokine, if at all, are far from clear (40).

Nontransgenic C5⁻ mice make very strong antibody responses to C5 dominated by a Th2-dependent isotype, yet their transgenic littermates respond with a default reaction to Th1 phenotype, precluding an antibody response. What is the underlying mechanism for this? We have no final answer to this at present, but propose that the phenomenon is due to a gross imbalance in the frequencies of antigen-specific T cells compared with antigen-specific B cells. Dendritic cells capable of presenting C5 are more frequent than antigen-specific B cells, so that their chances to encounter a C5-specific T cell are increased. This will result in a strong bias to Th1 development, which, through the release of IL-12 and IFN- γ , will negatively influence Th2 differentiation of those T cells that have been presented with antigen by B cells. The fact that there is an IgM antibody response indicates that there is help produced. The developmental state of T cells providing help for IgM is not known, but it is CD40 independent (41, 42) and may be Th0. Recent data (43) have suggested that Th2 development might require repeated signaling over a short time period. This could mean in our system that it takes a certain number of B–T interactions to sustain a switch to Th2, a situation that is guaranteed in the *in vitro* set up and in the adoptive transfer situation, but not under normal conditions in the transgenic host.

An interesting point worth mentioning is the development of IgG2a antibodies in the adoptive transfer studies in which presentation was targeted to B cells. The switch to

IgG2a is dependent on IFN- γ (44, 45). However, in transgenic mice, which generate IFN- γ in response to antigen, no IgG2a antibody response is observed until Th2 responses are present. There is an observation that the in vitro IFN- γ -mediated IgG2a antibody response is dependent on the presence of Th2-type cytokines such as IL-5 (46), and our data in the transgenic system support this suggestion.

Taken together, our data suggest that the B cell is the crucial APC for the development of Th2 responses. A crucial molecule involved in this process is CD40 on the sur-

face of B cells. Whether this is sufficient or whether there are additional signals from other cell surface molecules involved remains to be determined. The demonstration that B cell presentation can override an intrinsic propensity for Th1 development in this system opens the possibility to deliberately manipulate immune responses for the desired effector class. A very recent example of this is the finding that targeting of myelin basic protein to B cells abrogates induction of experimental allergic encephalomyelitis and results in switching to IL-4 secretion (47).

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