

Antigen-pulsed Dendritic Cells Can Efficiently Induce an Antibody Response In Vivo

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Summary

The aim of this study was to develop an immunization procedure avoiding external adjuvant. Data are presented showing that syngeneic dendritic cells (DC), which have been pulsed in vitro with antigen, induce a strong antibody response in mice. By contrast, antigen (Ag)-pulsed low-density B cells, although equally able to induce interleukin 2 secretion by an Ag-specific T cell hybridoma in vitro, only weakly prime the mice in vivo. Moreover, we show that the injection of Ag-pulsed DC induces the synthesis of isotypes similar to the immunoglobulin classes detected after immunization with the same Ag in complete Freund's adjuvant. Importantly, high amounts of IgG2a antibodies are produced, suggesting that T helper type 1 cells are activated. Collectively, these data indicate that DC can initiate a primary humoral response and that they may be used as physiological adjuvant in vivo.

The induction of immune responses in vivo is typically performed with antigens administered in artificial adjuvants, like alum and CFA (1). However, the discovery of dendritic cells (DC)¹ (2), which most efficiently activate a primary T cell response in vitro (3), suggests that these cells could be used to induce antibody responses in situ, avoiding the use of toxic adjuvants. These cells have some unique properties in vivo, as compared with the other APC: they are widely distributed in lymphoid as well as in nonlymphoid tissues (reviewed in reference 4); they seem to be the major source of processed antigen in vivo (5); and they home to the T-dependent region of lymph nodes and spleen (6, 7).

DC appear to play a major role in initiating various T cell immune responses in vivo, such as contact sensitivity (8–10), allograft rejection (11–14), and activation of MHC-restricted T cell responses (15). Taken together, these results suggest that injection of in vitro pulsed DC may provide an efficient way for inducing T-dependent immunity without the use of external adjuvant.

Little is known, however, about the induction by DC of specific B cell responses in vivo. Indeed, although it has been shown in our laboratory that mouse dendritic cells, pulsed in vitro with tobacco mosaic virus, could strongly enhance the primary and the secondary responses to the virus (16),

similar data could not be obtained with other antigens like soluble proteins.

Recent studies (15, 17) clearly showed that the capacity of the DC population to process and present proteins was downregulated when the dendritic cells matured in culture. In this report, we show that injection of DC appropriately pulsed in vitro with soluble protein antigens induces strong specific humoral responses in vivo.

Materials and Methods

Mice. Female DBA/2 mice (H-2^d), 6–8 wk old, were purchased from Charles River Wiga (Sulzfeld, Germany).

Antigens. The antigens used in this study were Myoglobin from sperm whale skeletal muscle and gamma-globulin from human blood, fraction II (both from Fluka Chemie AG, Buchs, Switzerland).

Culture Medium. The complete culture medium used in all experiments was RPMI 1640 (Seromed; Biochem KG, Berlin Germany) supplemented with 10% FCS (Byosis S.A., Compiègne, France), penicillin, streptomycin, nonessential amino acids, sodium pyruvate, 2-ME and L-glutamine (Flow ICN Biomedicals, Buxs, UK).

T Cell Hybridoma. The I-E^d-restricted, myoglobin-specific hybridoma 13-26-8-HG.1 was derived by Dr. A. Livingstone (Basel Institute of Immunology, Basel, Switzerland).

Antigen-presenting Cells. Spleens were digested with collagenase (CLS III; Worthington Biochemical Corp., Freehold, NJ) and sepa-

¹ Abbreviations used in this paper: Ag, antigen; DC, dendritic cell; HGG, human gamma globulin.

rated into low and high density fractions on BSA gradient (Bovuminar Cohn fraction V powder; Armour Pharmaceutical Co., Tarrytown, NY). The spleen DC were purified according to a procedure described by Crowley et al. (18). Briefly, low-density cells were cultured during 2 h in 10% FCS-containing medium and the nonadherent cells were removed by vigorous pipetting. The same procedure was repeated with a shorter (1 h) incubation without FCS. After overnight culture, nonadherent cells contain at least 90% of dendritic cells (as assessed by morphology and specific staining). To obtain low-density B cells, the nonadherent cells obtained after the 2-h incubation (before vigorous pipetting) were depleted of T cells. For convenience, these low density, nonadherent, T-depleted spleen cells will be called low-density B cells.

Antigen Pulsing of APC. For dendritic cell pulsing, the adherent cells of the low-density fraction of spleen were cultured overnight in complete medium containing 100 $\mu\text{g/ml}$ of antigen. The nonadherent cells were then collected and were mainly DC (referred to as antigen [Ag]-pulsed DC). The low-density B cells were cultured in the same conditions and all cells were collected after overnight culture.

IL-2 Production by T Cell Hybridoma. The T cell hybridoma was cultured with varying numbers of APC in the presence of intact protein in complete culture medium in 96-well flat-bottomed microtiter plates. After a 24-h culture period, the supernatants were assayed for IL-2 using an IL-2-sensitive subline of the CTLL cell line (19). A total of 10^4 cells were incubated with the supernatants and 0.5 μCi [^3H]TdR was added per well during the final 6–16 h of culture. Cells were harvested, and incorporation of radioactivity was assessed as described above.

Determination of Antigen-specific Antibody Levels. Serum levels of antigen-specific antibodies were determined by ELISA according to standard procedures using polyclonal goat anti-mouse IgG reagent (Boehringer Mannheim Biochemicals, Mannheim, Germany) or isotype-specific rat mAbs (20). Antibody titers were calculated based on linear regression analysis of the optical densities. Results are expressed as titers determined using the midpoint of the titration curves relative to an internal standard run in each assay.

Immunization Protocols. For primary response, mice received an intravenous injection of 3×10^5 Ag-pulsed syngeneic DC. Control mice were injected with unpulsed DC, Ag-pulsed low-density B cells, or were left untreated. 5 d later, all mice received a boost of 100 μg of soluble antigen intravenously, except one group of untreated mice which was injected intraperitoneally with 100 μg of antigen emulsified in CFA. All mice were bled 7 or 8 d after the antigen boost. For secondary response, all groups of mice received an injection of 100 μg of soluble antigen intravenously 1 mo after the initial treatment and were bled 7 d later.

Results

Fresh Splenic Dendritic Cells Present Native Proteins In Vitro.

The initiation of any T cell response requires two independent steps: Ag presentation and T cell sensitization. Ag presentation generates the ligand that is recognized by the α/β heterodimer of the clonally specific portion of the TCR for Ag, and usually requires the generation of small peptides. Although it is clear that purified splenic DC have a poor, if any, capacity for processing, data from Romani et al. (17) clearly showed that handling of intact proteins was down-regulated in cultured as compared with fresh epidermal Langerhans cells. More recently, Inaba et al. (15) showed that fresh splenic DC were able to process native proteins early, i.e.,

during the purification procedure of the DC. In a preliminary experiment, we compared the ability of dendritic cells and control cells to process and present myoglobin in vitro. We used a T cell hybridoma, since its activation only requires TCR occupancy, i.e., the presence of the appropriate antigen in the context of self MHC, and does not depend on any costimulatory signal (21). As control APC, we chose cells that were isolated from the same low-density fraction as the dendritic cells, but were nonadherent during the 2-h culture and were depleted of T cells (see Material and Methods). Fig. 1 shows that both APC populations (DC or low-density B cells), pulsed during overnight culture, strongly induce IL-2 secretion by a myoglobin-specific T cell hybridoma. The two types of APC, however, have distinct properties. Indeed, 24-h-old, purified DC cultured with antigen only slightly induce the activation of the T cell hybridoma, whereas 24-h-old low-density B cells very efficiently present the antigen in the same conditions.

Dendritic Cells Pulsed In Vitro with Antigen Induce a Specific Humoral Response In Vivo. To evaluate the ability of DC to induce an in vivo humoral response, syngeneic DC were pulsed with myoglobin during overnight culture and 3×10^5 cells

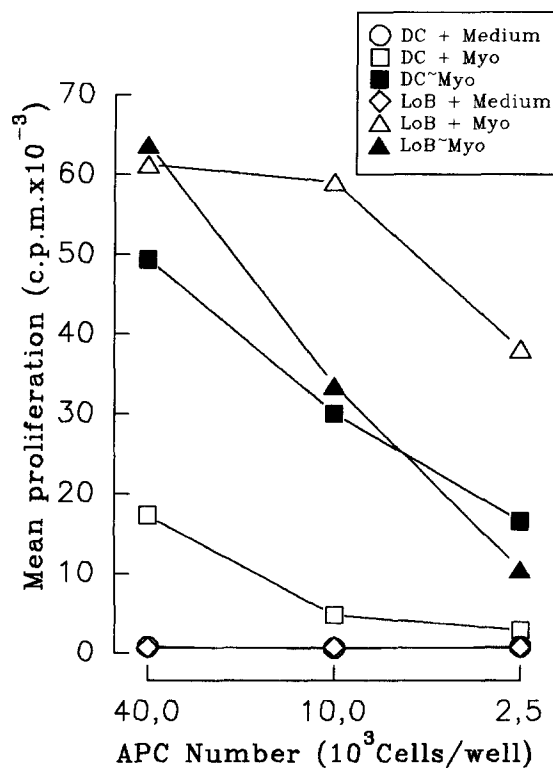


Figure 1. Fresh DC process native antigen in culture. Fresh DC and low-density B cells were cultured overnight in complete medium containing myoglobin (DC~myo and LoB~myo). Cells were washed, counted, and cultured with 3×10^4 T-T hybridoma cells (13-26-8). 24-h culture supernatants were assayed for IL-2 production in response to antigen-specific stimulation. Control APC (DC and LoB) were cultured without Ag during the purification steps and were cultured with hybridoma cells with (+ Myo) or without (+ medium) addition of antigen. Three experiments were performed with similar results.

were injected intravenously into syngeneic DBA/2 mice. 5 d later, animals were injected with 100 μ g of antigen in saline. Control groups included mice injected with soluble antigen only, and mice that received unpulsed DC and the antigen

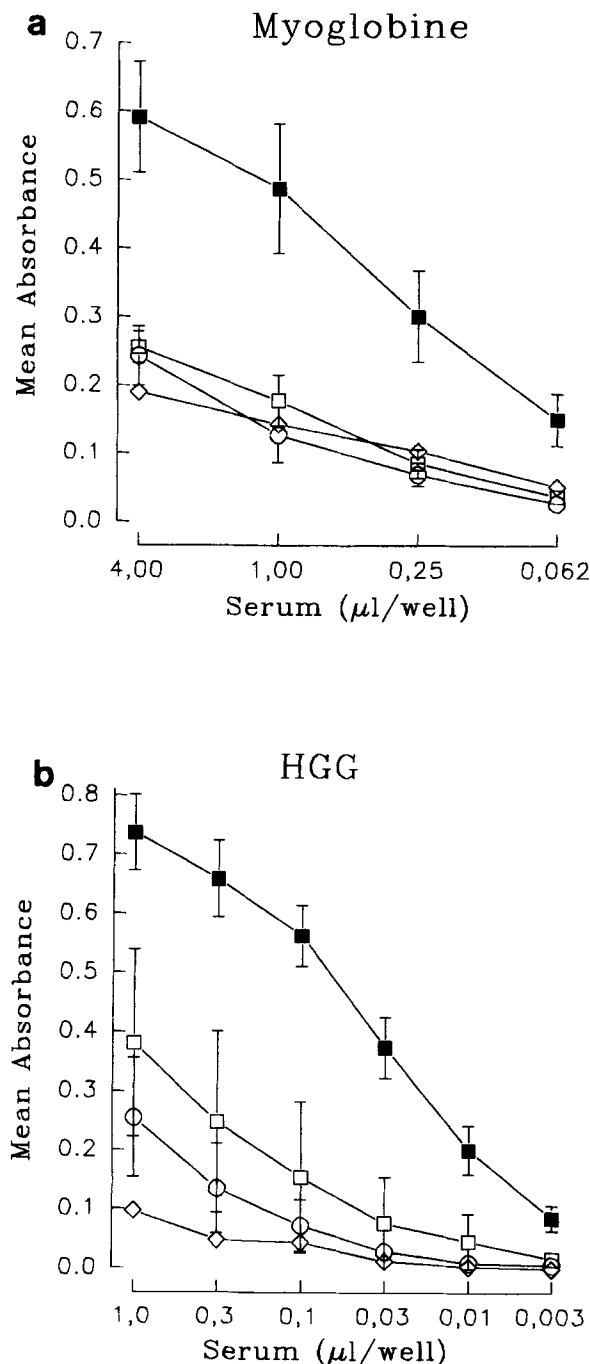


Figure 2. Antigen-pulsed DC induce a primary humoral response in vivo. DBA/2 mice (at least five per group) were injected with 3×10^5 myoglobin-pulsed (a) or HGG-pulsed (b) DC and boosted with 100 μ g of the same antigen in saline (closed squares). Control groups included untreated mice (diamonds), mice injected with unpulsed DC (open squares), and mice that only received soluble Ag (circles). All mice were bled 8 d after antigen boost, and antigen-specific antibodies were measured in individual sera using a goat anti-mouse Ig reagent.

boost. The data in Fig. 2 a show that in vivo administration of Ag-pulsed DC induced a strong humoral response upon challenge with soluble antigen, whereas control mice produced little specific antibodies. Similar data were obtained with human gamma globulin (HGG): DBA/2 mice injected with HGG-pulsed DC and boosted with the same antigen in saline synthesized high amounts of antibodies specific for HGG (Fig. 2 b).

The Isotype Pattern Reflects the Activation of Th1 Cells In Vivo Recent studies indicate that unique cofactors are necessary for the selective activation of cloned murine CD4⁺ cells representing Th1 and Th2 cells and that these factors are produced by specialized APC populations (22). Since the regulation of isotype switching in vivo is dependent upon the activation of different types of T helper cells (23), we investigated the class distribution of the specific antibodies. Fig. 3 summarizes the isotypes of the myoglobin-specific antibodies from mice tested individually in two independent experiments. The data show that high concentrations of IgG1 and variable but elevated amounts of IgG2a are secreted in the primary and secondary responses of DBA/2 mice primed with antigen-pulsed syngeneic DC. The injection of Ag-pulsed low-density B cells induces a low primary response, but the level of specific antibodies is increased after the antigen boost. Similar data were obtained in three independent experiments for the primary and secondary responses specific for HGG: Fig. 4 shows that HGG-pulsed DC are as potent as CFA in inducing specific antibody responses of IgG1 and IgG2a isotypes.

Discussion

The major observation from this study is that syngeneic DC that have been pulsed in vitro with native proteins induce a strong specific B cell response in vivo in unprimed animals that are boosted with soluble antigen. Thus, extracorporeal pulsing of DC may provide a physiological pathway for inducing T-dependent humoral responses in vivo.

The priming of naive animals by using elements of the immune system itself offers several advantages. It avoids the toxicity and the nonspecific immune activation often associated with the use of artificial adjuvants. Moreover, DC appear to very efficiently generate the antigenic epitopes (24, 25) that can be presented by self-MHC, and finally, the injection of DC promotes a specific memory B cell response.

The potent "adjuvant" capacity of DC, as compared with low-density B cells, correlates with their unique properties in vivo (for review, see reference 4). In particular, by down-regulating antigen processing (15, 17; Fig. 1), DC may not displace the acquired antigen with other antigens or with self proteins. This property appears to confer on the DC some "fidelity" to the Ag (15; and T. Sornasse, data not shown).

Our results are in accordance with previous in vitro data showing that DC are required for the development of T-dependent antibody response by mouse and human lymphocytes in vitro (26, 27). In vivo, a number of experiments have shown that the B cell is the initiating APC in peripheral lymph nodes (28–30), whereas other data suggest that non-B cells, "professional" APC, are required to initiate an

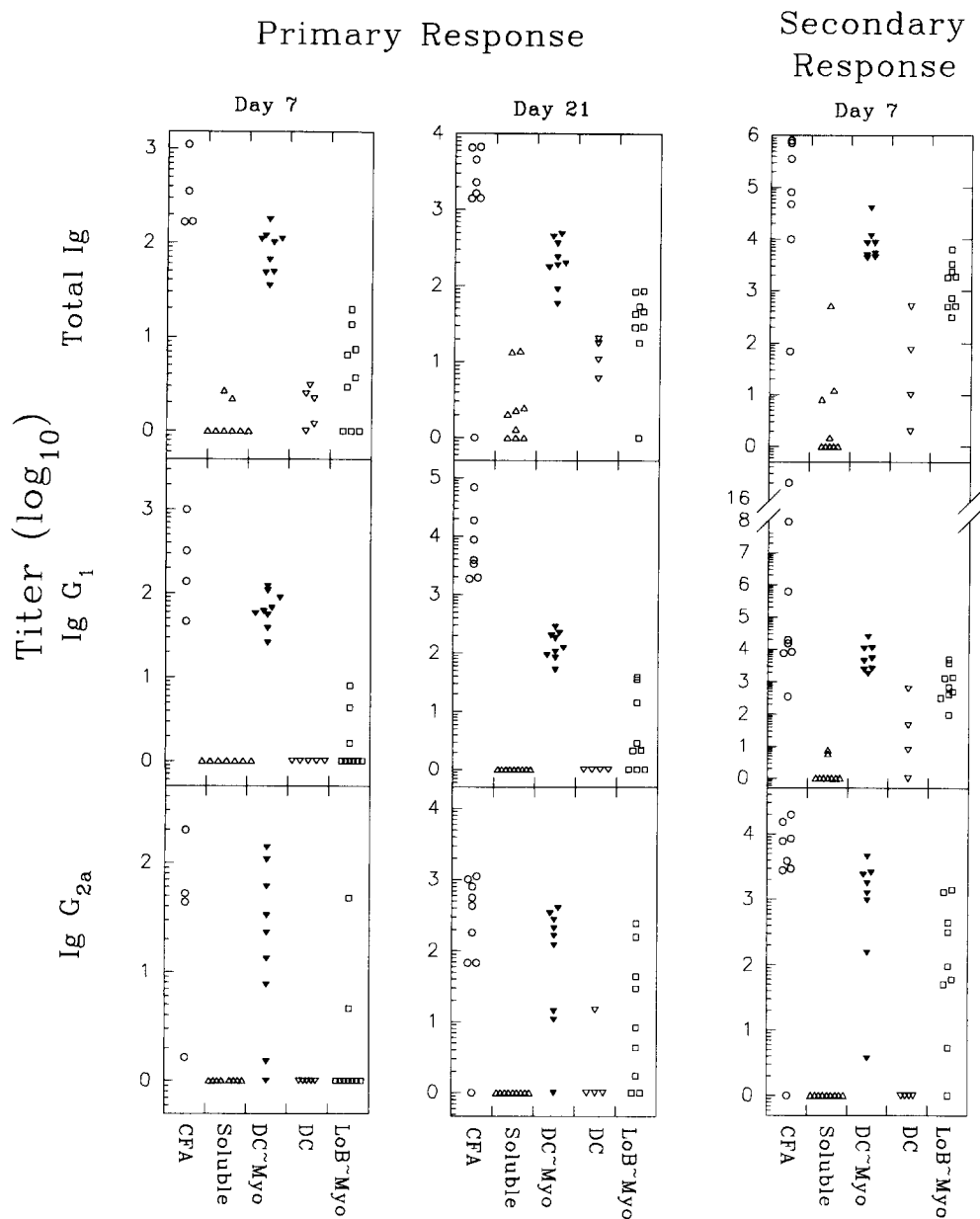


Figure 3. Isotype analysis of myoglobin-specific primary and secondary responses. Mice were injected on day 0 with either antigen-pulsed DC (DC~Myo), unpulsed DC (DC), or antigen-pulsed low-density B cells (LoB~Myo). All groups of mice received 100 μ g of soluble antigen on day 5. Control mice include one group that was injected with 100 μ g of myoglobin emulsified in CFA and one group that only received 100 μ g of soluble Ag on day 5. Total specific response (Total Ig), as well as specific antibodies of IgG1 or IgG2a isotypes, were measured as described in Materials and Methods.

anti-SRBC primary immune response (31). Our data emphasize the main role of DC in initiating primary responses in vivo.

Low-density B cells, purified from the same low-density floating fraction as DC, very efficiently present myoglobin to T cell hybridoma in vitro. Nevertheless, they only induce a weak primary B cell response in vivo as compared with DC. However, the antibody response induced by the injection of low-density B cells is significantly higher than the response observed after injection of soluble antigen. Thus, low-density B cells may contain a population of cells able to stimulate resting T cells and initiate a humoral response. These cells could be activated B cells, or alternatively could be contaminating DC, since we detect ~1–2% of DC in the preparation (data not shown).

When primed mice were boosted with soluble Ag, a sec-

ondary response of greater amplitude is observed (Figs. 3 and 4). These results demonstrate that priming with Ag-pulsed DC elicits a memory response.

The effect of DC on antigen-specific responses most likely involves helper T lymphocytes which, in turn, recognize MHC-compatible, antigen-stimulated B cells recirculating in the vicinity. Optimal sensitization of T cells requires two steps: an antigen-specific step, the occupancy of the TCR by Ag + MHC, and an Ag nonspecific step, the costimulatory signal delivered by the APC (32, 33). IL-1 seems to be a requisite costimulator for the growth of selected CD4⁺ Th2 clones, whereas optimal Th1 responses require a costimulatory signal that could be the murine B7 (34). Since splenic DC do not appear to secrete or express IL-1 (35), and since low-density splenic APC can replace the costimulator for Th1

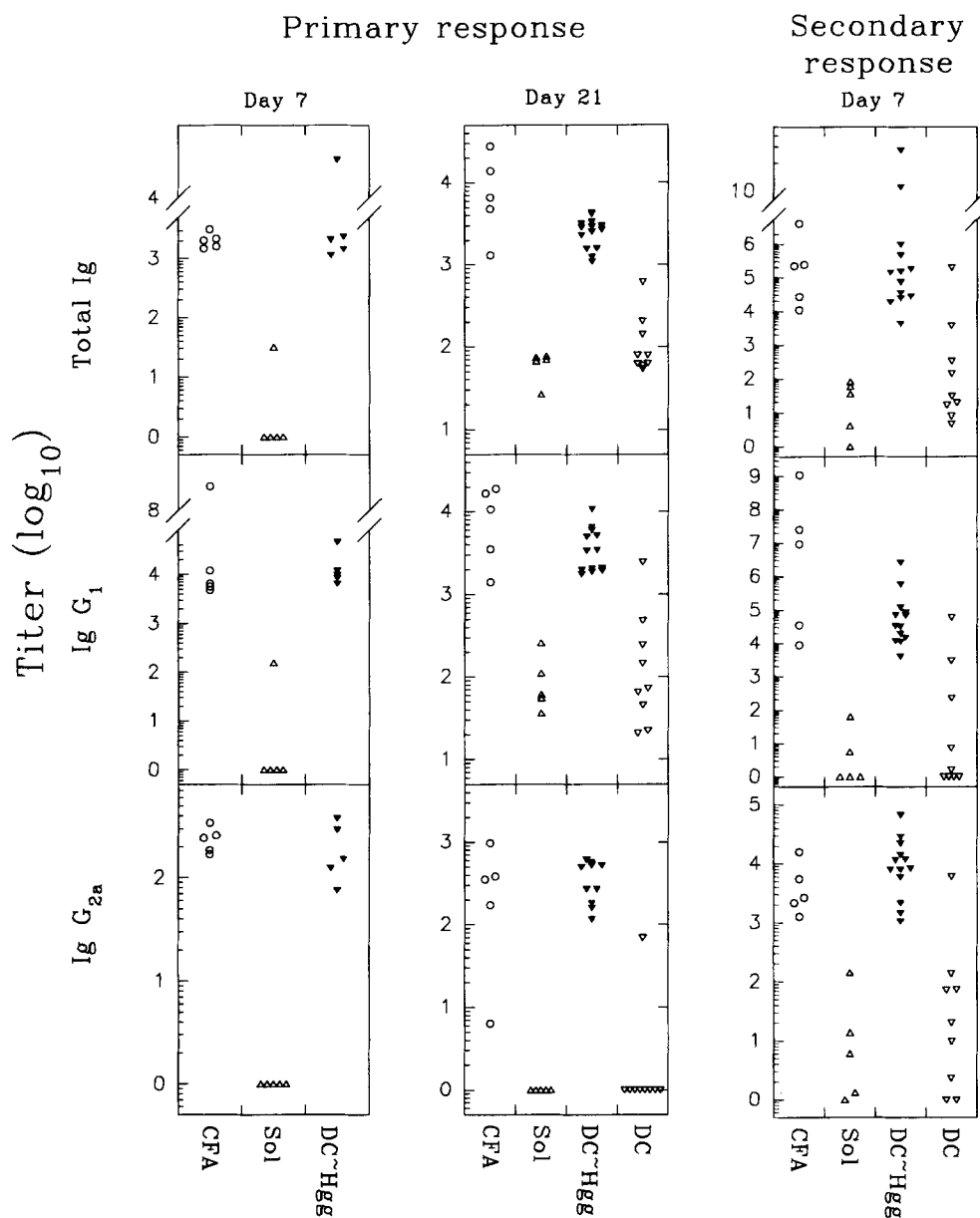


Figure 4. Isotype analysis of HGG-specific primary and secondary responses. Mice were either untreated (Sol), injected with antigen-pulsed DC (DC~HGG), or with unpulsed DC (DC). All groups were boosted with 100 μ g of soluble antigen 5 d later. A group of mice received 100 μ g of HGG emulsified in CFA (CFA). Total specific response (Total Ig), as well as specific antibodies of IgG1 and IgG2a isotypes, were tested as described in Materials and Methods.

cells (for review, see reference 36), it is tempting to speculate that injection of Ag-pulsed DC mainly activates Th1 cells in vivo.

The presence of IgG2a in most sera tested from mice primed with Ag-pulsed DC strongly supports this hypothesis. It has indeed been shown that clones of Th1 type specifically induced Ag-specific B cells to secrete IgG2a (37). Work is in progress to characterize the lymphokine pattern produced by Ag-specific, CD4⁺ T cells isolated from the mice primed with Ag-pulsed DC.

It is of note that the synthesis of specific antibodies of IgG2a

isotype is of physiological importance since this isotype has been shown to play a central role in the elimination of antigen in vivo (38, 39).

We think that Ag-pulsed DC could instruct a T helper lymphocyte, uncommitted in its lymphokine pattern, to differentiate into a Th1 type lymphocyte. The choice between Th1 and Th2 could therefore be due to the nature of the cell that presents antigen.

In conclusion, DC may be used as a physiological adjuvant to induce cellular (15) and T cell-dependent humoral responses in vivo.

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