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Dendritic Cells Interact Directly with Naive B Lymphocytes to Transfer Antigen and Initiate Class Switching in a Primary T-Dependent Response[1]

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Dendritic cells (DC) are thought to initiate Ab synthesis by activation of T cells, which then provide cytokine and cell-bound "help" to B cells. Here, we provide evidence that DC can capture and retain unprocessed Ag in vitro and in vivo, and can transfer this Ag to naive B cells to initiate a specific Ab response. The response is skewed with 4- to 13-fold higher titers of IgG than IgM, and the predominant subclasses of Ab produced in naive animals are those associated with Th2-type responses. Ag retention and the skew in class switching is a physiologic phenomenon because DC loaded with Ag in vivo and isolated 24 h later initiated a class-switched, Ag-specific Ab response in naive animals. In vitro studies confirmed that DC provide naive B cells with signals that are essential for the synthesis of class-switched Ab. Taken together, these observations show that DC have an important role in the initiation of Ab synthesis by direct interaction with B cells. *The Journal of Immunology,* **1998, 161: 1313–1319.**

In T-dependent Ab responses, dendritic cells (DC)[3] are thought to be involved in Ab synthesis by priming resting T cells to peptides derived from internalized protein Ags resulting in a cascade of costimulatory signals (1–11). These T cells are then able to respond to peptide presented by B cells on MHC class II (12) and can provide cell-bound and secretory signals to B cells (13–20), leading to Ab synthesis, class switching, and the development of germinal centers. Activation of B cells in a primary response occurs in T cell areas of the spleen (21), but whether DC have a role in this activation is not known. We have previously shown that ex vivo DC interact with naive B cells, independently of T cells, to form short-lived clusters, and such clusters have been seen in vivo (22). Here we show that in vivo DC can transport Ag, and that both in vivo and in vitro can transfer this Ag to naive B cells and give cell-bound signals to B cells that are required for subsequent class switching. The results suggest that the interaction between DC and B cells has an important role in the initiation and regulation of a primary Ab response.

Materials and Methods

Isolation of splenic DC

Spleens from 10- to 12-wk-old male SPF PVG rats were digested in collagenase, dispase, and DNase (Boehringer Mannheim, Lewes, U.K.; (23)). RBC were lysed and cells incubated with OX52 (pan T cell), OX8 (T cells and NK cells), OX12 (anti-light chain), OX33 (pan-B cells), and biotinylated anti-μ and anti-γ Abs (Binding Site, Birmingham, U.K.) for 1 h at 4°C. Labeled cells were depleted by rosetting with anti-mouse Ig-coated SRBCs and layering over Histopaque (Sigma, St. Louis, MO). Contaminating cells were depleted using the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany). The final DC-enriched population was examined by flow cytometry and immunocytochemistry and contained ~85% DC based on morphology and expression of MHC class II, with less than 1% T cells, B cells, or macrophages. The flow cytometry profile in Figure 1*A* shows the absence of cells expressing OX52 (T cells) or OX12 (B cells), with the majority of cells expressing MHC class II. Immunocytochemistry showed less than 1% macrophages or plasma/preplasma cells (Table I).

B cell isolation

B cells were isolated from rat spleens by disruption through a cell filter, followed by depletion of RBC with Gey's solution. The remaining cells were incubated in petri dishes for 1 h at 37°C to remove adherent cells (macrophages and fibroblasts), followed by treatment with 10 μg/ml OX52 (pan-T cell), OX62 (DC specific marker), anti-γ-biotin, and OX41 (macrophages and DC subpopulation) for 1 h at 4°C. Labeled cells were depleted by rosetting with anti-mouse Ig-coated SRBCs and layering over Histopaque (Sigma). Contaminating cells were depleted using the MACS system (Miltenyi Biotec). Flow cytometry profiles in Figure 1*B* show that more than 95% of cells are B cells that expressed IgM and low levels of MHC class II. Less than 1% of cells expressed OX52 (T cells), or moderately high MHC class II levels (DC). Immunocytochemistry showed <1% of macrophages or plasma/preplasma cells (Table I).

Flow cytometry

Cells were incubated with 10 μg/ml OX52, OX6 (MHC class II), OX12 (Ig light chain), or anti-IgM-biotin for 1 h at 4°C. Labeled cells were detected by goat anti-mouse IgG (rat adsorbed)-phycoerythrin (Serotec, Oxford, U.K.) or Streptavidin-Quantum red (Sigma).

Detection of macrophages

Opsonized SRBC were prepared by incubation of SRBC with rabbit anti-SRBC for 1 h at 37°C and washed in PBS three times. To detect macrophages in cell preparations, the opsonized SRBC were mixed with a sample of these cells for 1 h at 37°C, the red cells lysed, and the cells cytocentrifuged onto slides. The slides were stained with Giemsa stain and the number of cells that had ingested opsonized SRBC were scored as macrophages. DC show no evidence of uptake of opsonized SRBC.

Detection of preplasma and plasma cells

Samples of cell preparations were cytocentrifuged onto slides and fixed in cold ethanol. The cells were treated with OX12 for 1 h at room temperature followed by HRP-anti-Ig and substrate (diaminobenzidine and hydrogen peroxide, both from Sigma). While B cells were very weakly stained, plasma and preplasma cells had large quantities of cytoplasmic Ig.

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[3] Abbreviations used in this paper: DC, dendritic cells; B cells, B lymphocytes; KLH, keyhole limpet hemocyanin; HSA, human serum albumin; HRP, horseradish peroxidase; MACS, magnetic cell separation system; MFI, mean fluorescence intensity; SPF, specific pathogen-free; BMDC, bone marrow dendritic cells.

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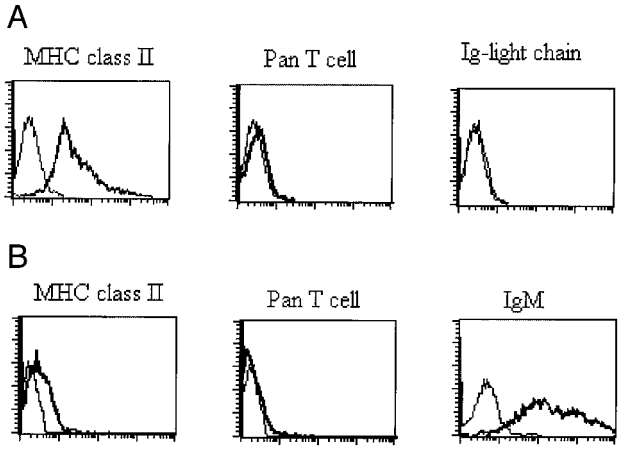

FIGURE 1. Flow cytometry profiles of DC and B cell preparations. *A*, representative profiles of DC preparations; *B*, representative profiles of B cells preparations.

In vitro Ag pulsing

DC were pulsed with 100 μg/ml keyhole limpet hemocyanin (KLH, Sigma) or DNP-KLH (Calbiochem, La Jolla, CA) for 1 h at 37°C and washed in PBS. To remove noninternalized Ag, cells were incubated in 0.02 M EDTA/PBS (24) for 5 min and given three washes in PBS before use in cultures or administration to rats. To confirm that EDTA removed surface Ag, we measured the mean flurocence intensity (MFI) of DC pulsed with fluoresceinated DNP-KLH at 4°C and 37°C, before and after treatment with EDTA. EDTA reduced the MFI of cells pulsed with Ag at 4°C to background levels, with negligible effects on the MFI of DC pulsed at 37°C, i.e., EDTA did not affect endocytosed Ag.

In vivo Ag pulsing

Rats were given 500 μg DNP-KLH i.v. and the DC isolated from their spleens after 4, 12, and 24 h.

In vivo immunization

A total of 1×10^6 DC pulsed in vitro with DNP-KLH (see above) were administered i.v. to groups of KLH-primed or unprimed PVG rats. To prime rats for KLH, approximately 5×10^5 KLH-pulsed DC were administered i.v. 24 to 48 h before immunization. Positive control rats were given 500 μg DNP-KLH i.v. Each group contained four rats and the Ab titers were assayed by ELISA at the time of immunization and after 7 and 14 days.

In vitro cell culture

Cells were cultured in Iscove's DMEM with 5% FCS (Life Technologies, Paisley, Scotland, U.K.), 2 mM glutamine (Life Technologies), 25 mM 2-ME and 45 μg/ml penicillin/streptomycin (Life Technologies). B cells and Ag-pulsed DC were cultured at a ratio of 10 B cells/DC at a cell density of 2.5×10^6 cells/ml in 24-well plates. After 20 h, DC were depleted by rosetting or MACS and B cells incubated with a DC-activated, KLH-specific T cell line. The isolated B cell population did not contain detectable numbers of DC as assessed by FACS or immunocytochemistry.

Table I. *Percentage of macrophages and plasma/preplasma cells in DC and B cell preparations*[a]

	No. of Cells	
	DC	B Cells
Uptake of opsinized SRBC	<1% (4/500)	<1% (2/500)
Cytoplasmic Ig (plasma/preplasma cells)	<1% (1/300)	3% (15/500)

[a] The number of contaminating macrophages was calculated by counting the number of cells that phagocytosed opsonized SRBC. The number of plasma and preplasma cells was calculated by counting cells with cytoplasmic Ig on cytospins.

Confocal microscopy

To localize Ag in DC, purified cells were pulsed with FITC-human serum albumin (HSA), treated with EDTA, and cultured. After 6, 12, 24, and 48 h, the cells were examined by confocal microscopy. To show retention of native Ag, cells were pulsed with 1 mg/ml horseradish peroxidase (HRP) for 1 h, cultured, and enzyme activity was detected by diaminobenzidine (Sigma) and HRP (Sigma). Images were obtained using a Bio-Rad (Richmond, CA) MRC 1000 confocal laser-scanning microscope. Images were collected sequentially (zoom ×8.0). For Figure 2*L*, a phase contrast image was obtained using the Bio-Rad transmission detector.

Ab measurement

Ab titers were determined as described by van Essen et al., using 100 μg/ml (for serum) and 500 μg/ml (for culture supernatants) DNP-HSA to coat plates (25). Curves of absorbance against serum dilution were plotted and the Ab titers were determined as the reciprocal dilution that gave an absorbance of 30% of the maximum absorbance reading for that particular assay. Ab titers shown are mean ± SEM.

Results

DC can retain native Ag

To show that DC can retain native Ag, ex vivo splenic DC were incubated with FITC-HSA or HRP, and prepared for confocal microscopy immediately or after 6, 12, 24, or 48 h in culture. No fluorescent Ag could be detected in DC immediately after pulsing, but by 6 h Ag was located in cytoplasmic vesicles dispersed through the cell. Figure 2 (*A–F*) shows equatorial optical sections of DC pulsed with FITC-Ag after 12 h and stained for MHC class I (Fig. 2, *A–C*) and MHC class II (Fig. 2, *D–F*) to determine the localization of Ag and the markers. Spleen DC varied in their morphology and distribution of class II molecules (Fig. 2, *D–K*). Some DC were large, very irregular cells with most class II molecules expressed on their plasma membrane (Fig. 2, *F*, *J*, and *K*) whereas others were less irregular with a lower level of class II expression on the cell surface and the majority of class II molecules contained in cytoplasmic vesicles (Fig. 2, *G–I*). These cells represent DC in different stages of maturation (26, 27). Those DC that contained large amounts of cytoplasmic MHC class II appeared to have endocytosed more Ag than those with much membrane class II expression. These figures show that although some degree of colocalization occurs between vesicles containing Ag and MHC class I or class II molecules, in all cells Ag was also present in vesicles not expressing these molecules. The uptake and retention of HRP enzyme activity confirmed that unprocessed Ag was retained in DC for at least 48 h. Figure 2*L* shows retention of HRP after 12 h.

Ag-pulsed DC can initiate a primary Ag-specific Ab response and induce class switching in vivo

To investigate the functional significance of Ag retention by DC, ex vivo splenic DC were pulsed with DNP-KLH, treated with EDTA to remove surface-bound Ag, and injected i.v. into naive or KLH-primed rats. The preimmune sera of these rats did not contain detectable anti-DNP or anti-KLH Ab. Both naive and KLH-primed animals produced similar titers of IgG anti-DNP Ab with 4- to 10-fold less IgM anti-DNP (Fig. 3*A*). Similarly, the anti-KLH response in naive rats was also predominantly IgG (Fig. 3*B*). KLH-primed animals produced better responses to KLH than naive animals, suggesting a secondary response to the carrier protein. KLH-primed rats were also given DC that had been lysed after pulsing with DNP-KLH, to provide an equivalent quantity of free Ag that would control for nonspecific release of Ag. These animals produced a little IgM anti-DNP Ab but no detectable total IgG anti-DNP, indicating a need for intact DC to transport Ag and skew the response toward IgG (Fig. 3*A*). Moreover, Ag-pulsed BMDC provoked IgG responses with no detectable IgM in naive animals

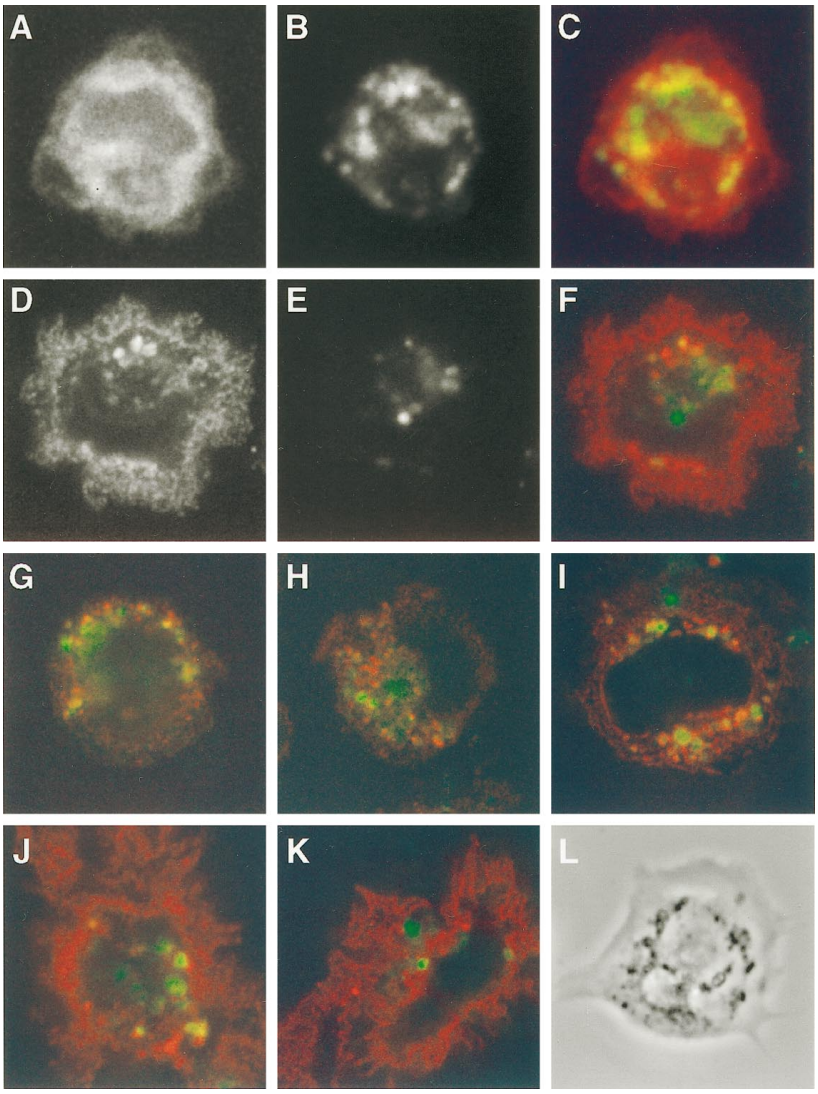

FIGURE 2. Confocal microscopy of DC pulsed with FITC-labeled-HSA or with HRP and stained for either MHC class I or class II (Texas Red or cy3) molecules after 12 h in culture. This figure shows the localization of (*A*) MHC class I, or (*D*) class II molecules in black and white. *B* and *E* show the localization of the FITC-Ag within the same cell in black and white. *C* and *F–K* show a merge of the two colors to show colocalization of the markers and FITC-HSA in a variety of cells. *G* to *K* show colocalization of FITC-HSA and class II molecules in DC. *L* shows vesicles containing HRP enzyme activity after 12 h in culture. (Magnification, ×320).

(data not shown). In comparison, naive animals given 500 μg free DNP-KLH i.v. gave much higher but similar titers of IgM and IgG of anti-DNP Ab (Fig. 3*C*), indicating the skew toward IgG was not dependent on the Ag.

Measurement of anti-DNP IgG subclasses showed that naive rats given Ag-pulsed, intact DC produced all four subclasses, with approximately 10-fold higher titers of IgG1 and IgG2a than IgG2b or IgG2c (Fig. 3*D*). IgG1 and IgG2a titers were similar in primed and naive animals given intact DC, while IgG2b and IgG2c levels were significantly higher in primed animals suggesting that switching to the latter two subclasses is enhanced by T cell priming. Moreover, very low titers of IgG2b, trace amounts of IgG2c, but no IgG1 or IgG2a, was detected in animals given lysed DC.

DC pulsed with Ag in vivo can retain Ag and initiate an immune response

To show that Ag retention and transfer to B cells was not an artifact of pulsing DC in vitro, we loaded DC with Ag in vivo. DC were isolated 4, 12, and 24 h after rats were given soluble DNP-KLH and injected i.v. into naive rats. These DC that had captured Ag in vivo initiated an anti-DNP Ab response in naive animals, which was again predominantly IgG with 4- to 13-fold less, or no, IgM (Fig. 4). The predominance of IgG anti-DNP Ab suggested that DC may have a direct role in class switching. These observations are not peculiar to KLH, since they could be reproduced with human serum albumin (Fig. 5). Furthermore, the IgG response observed in recipient rats could not be due to the transfer of B cells or plasma cells since such cells were not detected in significant numbers in the preparations transferred (Fig. 1 and Table I).

Early DC-B cell contact is required for class switching

To show that DC have a direct role in class switching, in vitro cultures were prepared as described in Figure 6. DC pulsed with DNP-KLH were cultured with naive B cells for 20 h, the DC depleted, and the recovered B cells cultured with KLH-primed T cells (Fig. 6*a*). To provide optimal help, the T cell line was cultured with KLH-pulsed DC before the addition of B cells. After 7 days, both IgG and IgM anti-DNP Ab was detected in the culture supernatants (Fig. 6*a*). To show that the class switch was dependent on early interaction with DC, B cells were cultured with soluble DNP-KLH for 20 h and then added to KLH-primed T cells (Fig. 6*b*). These cultures produced only IgM anti-DNP Ab (Fig. 6*b*). To show that contact between DC and B cells was essential, DC pulsed with DNP-KLH were cultured in transwells with B cells in a separate compartment (Fig. 6*c*). When these B cells were subsequently cultured with primed T cells, no IgG and only low titers of IgM anti-DNP Ab were detected (Fig. 6*c*). B cells cultured with DNP-KLH-pulsed DC in the absence of T cell help did not produce either IgM or IgG. These in vitro experiments demonstrate

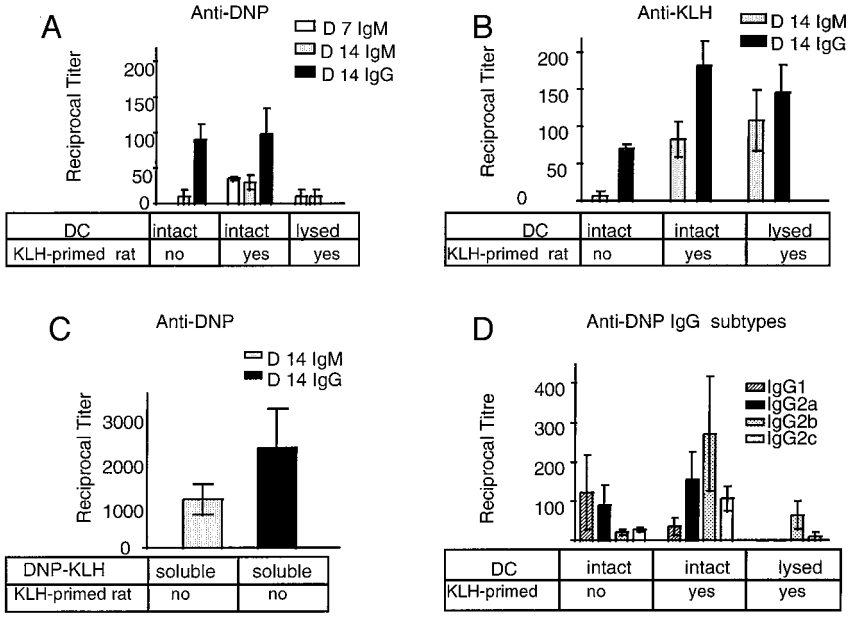

FIGURE 3. DC pulsed with DNP-KLH can initiate anti-DNP and anti-KLH Ab production in vivo, without additional soluble Ag. IgM and total IgG (*A*), anti-DNP and (*B*), anti-KLH Ab titers were measured after 7 and/or 14 days in the sera of naive and KLH-primed rats given intact or lysed DNP-KLH-pulsed DC. *C*, IgM and total IgG anti-DNP Ab titers in naive rats given soluble DNP-KLH. *D*, IgG subtypes of anti-DNP Ab in vivo, measured 14 days after the administration of whole or lysed DNP-KLH-pulsed DC to naive or KLH-primed rats.

that contact between DC and B cells is essential for efficient transfer of Ag to B cells and that DC provide B cells with an early signal that is essential for subsequent switching to IgG. However, B cells will not produce Ab without T cell help.

Discussion

B cell activation in a primary immune response requires recognition of native Ag. B cell activation occurs in T cell areas (21), but it has never been clear how Ag in peripheral tissues gains access to recirculating B cells in these areas. Ag diffuse via lymph or blood into these areas, but this mechanism would be inefficient for low Ag concentrations. Here, we identify a mechanism that targets Ag directly to naive B cells and facilitates their interaction with T cells. We show that DC can acquire Ag in the spleen, can retain it for at least 48 h (in a native form), and can present it to B cells in vitro and in vivo. In addition, we show that these DC give signals to B cells that influence the isotype of Ab they subsequently secrete.

Functional significance of Ag retention by DC

It is well recognized that DC acquire Ag in vivo and subsequently present peptides to naive T cells (1–11). Moreover, it has been widely assumed, by analogy with macrophages (28), that all native Ag would be rapidly broken down following endocytosis. However, if DC can present Ag to B cells, they must retain it in its native form for long enough to permit its transport from the periphery to secondary lymphoid tissues. This study shows that i.v. injection of Ag-pulsed splenic DC into naive rats stimulates the secretion of Ag-specific Ab within 7 days, showing that such DC can retain Ag long enough for it to be presented to B cells. The titers of the anti-DNP Ab resulting from the administration of DNP-KLH-pulsed DC were much lower than rats given soluble DNP-KLH. This reflects the minute quantity of Ag transported by DC and that only B cells that meet the DC will encounter Ag. When rats were injected with the same number of lysed Ag-pulsed DC, the titers were barely above background, demonstrating that DC can act as adjutants for small quantities of Ag.

We estimated the kinetics of Ag retention by DC in two ways. First, DC were pulsed in vitro with HRP, and active enzyme could be detected for at least 48 h, showing that some native Ag is retained for this period. To show that this was not just an in vitro phenomenon, we assessed Ag retention by DC in vivo. We reasoned that if we could load splenic DC in vivo, we could test Ag retention in vivo by transferring the DC to naive recipients and assess Ab synthesis. If the recipients made specific Ab this would show unequivocally that DC had retained Ag in a native form and that transfer to B cells was physiologically relevant. The results show that DC retain native Ag for 24 h in vivo (maximum period tested), as well as the time taken to transport Ag from the site of

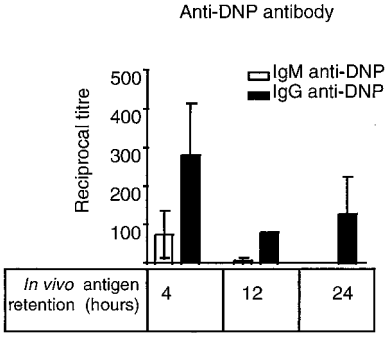

FIGURE 4. DC can retain unprocessed DNP-KLH in vivo. DC were isolated 4, 12, and 24 h after rats were given soluble DNP-KLH i.v. and transferred to naive rats. The IgG and IgM anti-DNP response in the naive rats was measured after 14 days.

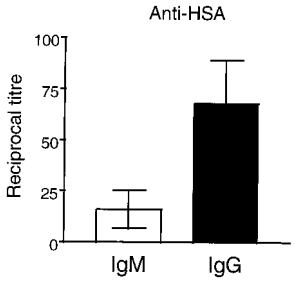

FIGURE 5. DC can retain unprocessed HSA in vivo. DC were isolated 24 h after rats were given soluble HSA i.v. and transferred to naive rats. The IgG and IgM anti-HSA response was measured after 14 days.

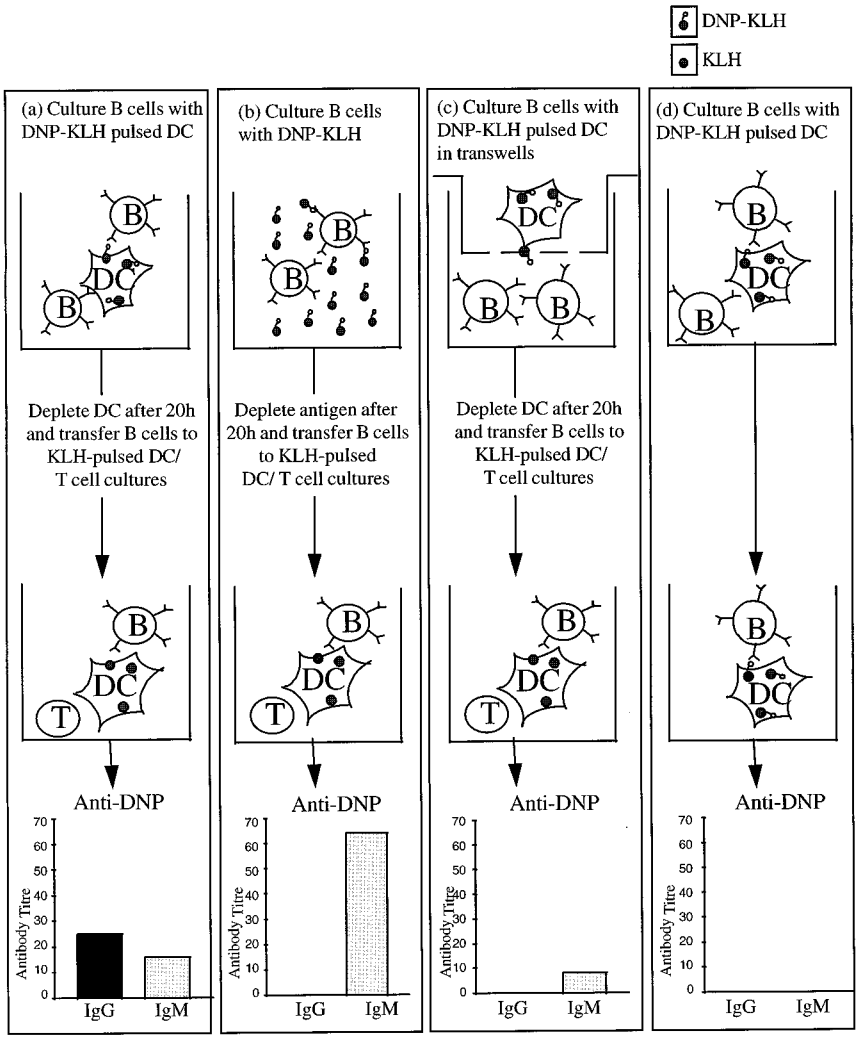

FIGURE 6. Direct contact between DC and B cells is required for switching to IgG. IgM and IgG anti-DNP Ab production was measured in culture supernatants after B cells were incubated with either (*a*) DNP-KLH-pulsed DC in direct contact, (*b*) free DNP-KLH, or (*c*) DNP-KLH-pulsed DC isolated in transwells, and then incubated with primed T cells. (*d*) Cultures with no T help were used as a negative control. Data represent one of four independent experiments that gave similar results.

injection to the spleen and to interact with B cells. Furthermore, confocal microscopy showed that some vesicles containing Ag did not contain MHC class II or class I molecules. These vesicles could be detected for at least 48 h, and although the presence of fluorescence cannot be directly equated with the retention of native Ag, this observation correlates well with HRP retention. Indeed, immature DC have been shown to possess mildly acidic compartments that store Ag for 24 h (29).

We do not know how Ag is released from DC to be recognized by B cells, but it has been shown that human DC can retain HIV in an internal compartment and, subsequently, this HIV can infect T cells adhering to the DC (30). We suggest that HIV may occupy the compartment used for Ag retention, and that the virus may have "hijacked" this mechanism to escape lysosomal enzymes and be subsequently released.

A role for DC in isotype switching by B cells

The regulation of B cell isotype switching in T-dependent responses is complex and requires both cell-to-cell signaling and cytokine recognition by B cells. CD40-CD40L interactions are essential for switching as shown by the absence of switching in patients with the X-linked hyper-IgM syndrome, in whom the CD40L gene is mutated (31), and in CD40 knockout mice (25). Studies in vitro have shown that, as well as CD40-CD40L interactions, T cell cytokines are also essential for switching (13–20, 25, 32–40). However, some observations suggest that this picture is still incomplete. For example, a study of the dynamics of a primary response found that switching from IgM to IgG was initiated by day 2 of the response and was maximal by days 6 to 8 (41). It was assumed that cytokines induce isotype switching very early in the immune response. Moreover, during B cell activation, transcription of unarranged heavy chain C region genes precedes switch recombination and is independent of cytokines (42). These observations suggest that B cells receive as yet unidentified signals that occur very early in the response, possibly before interaction with T cells.

In a primary Ab response, IgM secretion precedes IgG. We observed that rats immunized with Ag-pulsed BMDC or splenic DC consistently produced much higher levels of specific IgG than IgM. Indeed, with BMDC it was frequently impossible to detect an IgM response. To show that the skew toward IgG was not dependent on T cell priming, we compared the response of naive and KLH-primed rats to DNP-KLH-pulsed DC. KLH-pulsed DC were used for priming T cells to minimize free Ag in the rats, which could complicate the interpretation. In both naive and KLH-primed animals, the anti-DNP response was predominantly IgG. However, whereas anti-KLH responses in naive rats were predominantly IgG, KLH-primed animals produced higher total levels of anti-KLH Ab with relatively higher levels of IgM. To explain this apparent anomaly of higher IgM levels, we propose that the first injection of KLH-pulsed DC recruited Ag-specific recirculating T and B cells to the spleen, and initiated Ab synthesis with a skew

toward IgG (as for naive rats in Fig. 3*B*). When the recruited lymphocyte population was given a second dose of KLH on DNP-KLH-pulsed DC, they gave a better Ab response. The IgM response was also higher since T cell help for IgM production was readily available and, as soon as the DC provided Ag, IgM anti-KLH could be synthesized. Previous recruitment of KLH-specific T cells and B cells may also explain why the small amount of DNP-KLH released from lysed DC was able to stimulate significant levels of IgM anti-KLH Ab but very little IgM anti-DNP Ab (Fig. 3, *A* and *B*).

The consistent observation that rats given Ag pulsed-DC produced mostly IgG suggested that DC might have direct involvement in isotype switching. Since this switch occurred without additional soluble Ag, we proposed that DC might provide such a signal soon after Ag transfer, before Th cells had been recruited/activated. To investigate this possibility, we developed an in vitro model of Ab synthesis in which B cells were first cultured with Ag-pulsed DC, purified, and subsequently cultured with activated Th cells. We showed that B cells that had acquired DNP-KLH from DC secreted both IgM and IgG anti-DNP when given T cell help. In contrast, B cells cultured with free DNP-KLH (without DC) secreted only IgM when subsequently cultured with T cells under identical conditions. This shows clearly that Ag-bearing DC can transmit signals involved in isotype switching to Ag-specific B cells via direct contact. Contact is essential because when DC and B cells were separated by a transwell, no switching was seen when the B cells subsequently received T cell help. The Ab titers in these in vitro microcultures were low compared with in vivo titers, but we consider that this reflects the small proportion of Ag-specific B cells present in in vitro cultures, where specific recruitment is not possible. Moreover, Ag-specific B cells may not expand to the same levels in vitro, in the absence of in vivo microenvironment.

The molecular basis of DC-B cell signaling is not known. Direct CD40 signals to B cells are required for class switching since B cells from CD40 knockout mice do not switch (25). Since human DC have been shown to express CD40 ligand (43), it is possible that DC may provide CD40 signals to B cells. However, a study by Banchereau and colleagues showed that human B cells given CD40 signals (to replace T cells) and cytokines (IL-10 and TGF-β) did not switch to IgA without dendritic cells (44). This study shows that a CD40 signal was insufficient to isotype switch B cells and that another signal was provided by DC. Furthermore, a murine CD40 fusion protein did not inhibit subsequent switching when added to rat DC-B cell cultures (data not shown). This could signify that CD40 plays no role in the DC-B cell interaction, or that murine CD40 does not cross-react with rat.

DC also influence the subclass of IgG secreted after immunization. Moser and colleagues (45, 46) have shown that the type of APC (B cells, macrophages, or DC) used to immunize mice could affect the subclass of Ab produced. These differences were thought to reflect differences in T cell priming since the animals were subsequently given soluble Ag. Here we show that intact DC are essential for IgG1 and IgG2a production, and previous T cell priming is required for IgG2b and IgG2c. In rats, IgG1 and IgG2a represent Th2 responses whereas IgG2b is a Th1 Ab (47–51). Since all four subtypes are secreted after immunization with Ag-pulsed DC, we suggest that DC provide "switch" signals, but particular cytokines drive the expansion of switched B cells. DC may prime B cells to solicit Th2 cytokines from T cells to drive the switched phenotype. Studies have found that while T cells primed by DC produce Th1 cytokines, the addition of B cells results in Th2 cytokine production (52–54).

In conclusion, our results show that DC have important roles in the initiation and regulation of Ab synthesis, quite apart from their function in T cell activation. They acquire and retain protein Ags in an intact form for long enough to permit their migration to secondary lymphoid tissues and delivery to naive, recirculating, Ag-specific B cells. At the same time, they give signals to these B cells that modulate isotype switching and that can influence the subclass of IgG that is secreted. These results suggest that DC have complex roles in the regulation of humoral immune responses.

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