B Lymphocytes In Vivo Fail to Prime Naive T Cells but Can Stimulate Antigen-experienced T Lymphocytes

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Summary

The ability of B cells or macrophages and dendritic cells (DC) to elicit class II–restricted T cell responses in vivo was compared using a mouse chimera model. Severe combined immunodeficient (SCID) mice (H-2^d), reconstituted either with T or T+B lymphocytes from (H-2^d × H-2^b) donors, were immunized subcutaneously with protein antigen (Ag) to induce a class II–restricted T cell response. The frequency and major histocompatibility complex restriction of the resulting Ag-specific T cells were analyzed to establish whether B cells were necessary for the induction of class II–restricted T cell responses, and to determine the cell type on which priming had occurred. The results indicated that: (*a*) B cells are not necessary for the induction of a class II–restricted T cell response in vivo, as the frequencies of interleukin 2 (IL-2)- or IL-3-secreting T cells induced in the presence or absence of B cells were comparable. (*b*) Activation of naive T cells requires presentation of Ag on DC; Ag presented only on B cells is not sufficient to elicit a response. No H-2^b-restricted, IL-3-secreting cells could in fact be detected in SCID mice reconstituted with naive (H-2^d × H-2^b) T cells and nonimmune or antigen-primed (H-2^d × H-2^b) B cells. (*c*) Previously primed T cells are able to be stimulated by Ag presented by both B cells and DC. H-2^b-restricted, IL-3-secreting cells could in fact be readily demonstrated in SCID mice reconstituted with antigen-primed (H-2^d × H-2^b) T and B cells. Irrespective of whether the T cells were naive or previously activated, B cells were able to respond with an Ag-specific immunoglobulin G response, indicating that B cells were functional and able to present Ag in order to receive specific T cell help. Therefore, it appears that B cells are not necessary and do not participate in the initial priming of T cells; however, Ag presented by B cells can reactivate previously primed T cells. Taken together, these data indicate that during the course of an immune response Ag is first presented to naive T cells via DC, and only subsequently primed T cells can be stimulated by Ag presented by B cells.

The mechanisms by which APC take up, degrade, and reexpress processed Ag at the cell surface in association with class II MHC molecules are being increasingly clarified (reviewed in reference 1). However, despite considerable progress in defining the process at the cellular level, the identity of the cells that stimulate T cells in vivo is still unclear. Several cell populations have the properties required to be potential in vivo APC, but evidence concerning their role in different steps of the immune response, or in response to Ag entering the organism in different forms, or via different routes, is still incomplete. This question is of considerable importance, as different APC may be able to regulate the immune response in a positive or negative fashion by delivering opposing signals to the T cells (2). Moreover, different APC express different accessory molecules (3), and may be able to induce different functions (e.g., secretion of different sets of lymphokines) in the responding T cells (4, 5).

A considerable number of in vitro studies show that B cells are potent APC for T cells (reviewed in reference 6), being able to use their surface Ig to take up Ag present in very low amounts in the medium (7, 8), internalize it, and process it so it can be presented (9). Less is known concerning the ability of B cells to stimulate T cell responses in vivo (10). Evidence in favor of B cell involvement in T cell responses in vivo comes from experiments performed by different groups using mice treated from birth with anti-μ antibodies (11–15). These animals show a virtually complete block of the development of B cells, and are unable to mount T cell proliferative responses to Ag administered subcutaneously (11–14). Local administration of B cells can reconstitute the response (14, 16), suggesting that B cells are required for the generation of a T cell response. In apparent contrast with these findings, Lassila et al. (17) used a chicken chimera model to show that antibody responses to SRBC could only be generated in the presence of spleen cells (as a source of "professional" APC) that were MHC matched with the B cells. This

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implied that B cells themselves were not sufficient for the initiation of a T cell response. Several reasons could account for the apparent discrepancy between these two sets of data: the method of assessment (T vs. B cell responses), and the type and location of Ag challenge (different kinds of Ag and different lymphoid organs involved).

An experimental model was therefore designed that makes use of SCID mice to study the role of B cells vs. dendritic cells (DC)[1] in the induction of a T cell immune response. SCID mice are unable to generate normal T or B cells due to a recombinase defect that prevents successful rearrangement of the Ig and TCR loci (18, 19), but does not affect the development or function of DC (20, 21). SCID mice (H-2^d, BALB/c background) were reconstituted with mature T or T+B cells from semiallogeneic (BALB/c × B6)F_1 (H-2^d × H-2^b) donors, and immunized with Ag in adjuvant in order to induce a T cell response. The contribution of B cells (or their products) could be examined in these mice by comparing T cell responses in animals reconstituted with or without B cells. Also, analysis of the MHC restriction of Ag-specific T cells in animals reconstituted with T+B cells shows whether the T cells have been primed on DC (of host origin; H-2^d), in which case the whole response should be H-2^d restricted, or on B cells (of donor origin; H-2^d × H-2^b), in which case the response should be at least in part H-2^b restricted. The results described here show that B cells are not necessary for the induction of a T cell response, and are in fact unable to prime naive T cells. They are, however, able to reactivate previously primed T cells, indicating a role for B cells in presenting antigen in secondary responses.

Materials and Methods

Mice. C.B-17/Icr *scid/scid* (SCID; Igh-1^b congenic BALB/c) mice were bred at the Basel Institute for Immunology (BII) from breeding pairs obtained through the courtesy of Dr. M. Bosma (Fox Chase Cancer Institute, Philadelphia, PA). Mice of ~12 wk of age were tested before reconstitution for leakiness of the SCID phenotype by evaluating serum IgM and IgG titers in Ouchterlony assay or ELISA. (BALB/c × B6)F_1 mice (cB6) were bred at the BII or obtained from Bomholtgård (Ry, Denmark). BALB/c *nu/nu* were from Iffa Credo (L'Arbresle, France); C57BL/6 (B6) *nu/nu* were from Bomholtgård.

Antigens. OVA (grade V) was purchased from Sigma Chemical Co. (St. Louis, MO); KLH was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Coupling of DNP to protein was performed by incubating the protein solution in 0.1 M borate buffer with 2,4-dinitrofluorobenzene (2,4 DNFB) (Sigma Chemical Co.) at 37°C for a few minutes; unbound DNFB was removed by dialysis. The degree of haptenization was calculated from absorbance values at 280 and 360 nm as described (22).

Antibodies and FACS® Analysis. The mouse mAbs Y-3P (anti-I-$A^{b,k}$; 23), MK-D6 (anti-I-A^d; 24), 14-4-4S (anti-I-$E^{d,k,b}$; 25), RS-3.1 (anti-Igh-6a; 26), and MARK-1 (mouse anti-rat k, 27), and the hamster mAb 2C11 (anti-CD3; 28) were purified on the Affi-Gel protein A MAPS II kit (Bio-Rad Laboratories, Richmond, CA) from culture supernatants according to the manufacturer's instructions. The rat mAbs GK1.5 (anti-CD4; 29), 2.43 (anti-CD8.2; 30), 6B2 (anti-B220; 31), F4/80 (anti-macrophage; 32), and 2.4G2 (anti-Fcγ receptor; 33) were affinity purified from culture supernatants on protein G–Sepharose (Pharmacia LKB, Uppsala, Sweden). The IgM mAbs J11d (rat, anti-HSA; 34) and 28-13-3S (mouse, anti-K^b; 35) were purified from culture supernatants by 40% ammonium sulphate precipitation and dialysis against 5 mM phosphate buffer; the precipitate was collected by centrifugation and resuspended in PBS. FITC labeling and biotinylation were performed as described (36). Second-step reagents (goat anti-mouse Ig-FITC, goat anti-mouse IgG-PE, goat anti-mouse IgM-PE, Streptavidin-FITC, Streptavidin-PE) were from Jackson ImmunoResearch Labs, Inc., West Grove, PA). Stainings were performed on 2–5 × 10^5 RBC-depleted cells in 100 μl PBS + 2% FCS in U-bottomed 96-well plates (Dynatech Labs, Embrach, Switzerland) for 10 min on ice. Optimal antibody concentrations were determined experimentally and ranged between 0.25 and 1 μg/well; the anti-FcγR mAb 2.4G2 was used to inhibit nonspecific staining. Analysis was performed on a FACScan® (Becton Dickinson & Co., Mountain View, CA). Instrument compensation was set in each experiment using samples stained with single-color reagents.

Reconstitution of SCID Mice. Nonirradiated SCID mice were reconstituted by intravenous injection in the lateral tail vein of 20–70 × 10^6 sex-matched purified T or T+B cells in PBS; the number of T cells in the inoculum was maintained constant in each experiment. For all mice, reconstitution of the peripheral lymphoid organs was tested at the time of death by fluorescent staining of thymus, lymph node, and spleen cell suspensions with H-2^b- and Igh^a-specific reagents.

Splenic cB6 T or T + B Cells. For transfer into SCID mice, splenic cB6 T or T+B cells were prepared as follows. Mechanically disrupted spleen cell suspensions were fractionated by centrifugation on Percoll (Pharmacia LKB) gradients to deplete low density DC; the dense 60–70% fraction (ρ = 1.076–1.088 g/ml) was collected. B cells were depleted by incubation with sheep anti-mouse IgG-coated Dynabeads (Dynal, Oslo, Norway) and magnetic adherence. The injected population contained between 40 and 60% T cells in different experiments; after B cell depletion the percentage of B220$^+$ cells was <1%.

Naive T Cells. These were prepared from the thymuses of 4-wk-old cB6 donor mice (37) by depletion with J11d mAb and magnetic beads. Briefly, thymus cell suspensions were adjusted at ~2 × 10^7 cells/ml, incubated in purified J11d (10 μg/ml) in medium for 30 min on ice, washed, incubated in a cocktail of MARK-1, Y-3P, 14-4-4S mAbs (each 10 μg/ml) for 30 min on ice, washed, incubated with sheep anti-mouse IgG-coated Dynabeads (Dynal) for 30 min at 4°C with constant rocking, and depleted by two rounds of magnetic adherence. The treatment was repeated until a satisfactory depletion was achieved. Typically, the resulting T cell population was 80–85% CD4 or CD8 single positive and 70–75% CD3 high.

Normal or Ag-primed B Cells. These were prepared as described above from the spleens of nonimmune cB6 mice, or cB6 mice immunized 4–6 wk previously by intraperitoneal injection of 100 μg OVA-DNP in alum. T cells were depleted by magnetic sorting by incubation in a cocktail of purified GK1.5 (anti CD4) and 2.43 (anti CD8) at 10 μg/ml, and sheep anti-rat Dynabeads (Dynal) in the presence of 1% BALB/c normal serum. The resulting population was 80–85% B220$^+$.

Ag-primed T and B Cells. These were prepared from the peripheral lymph nodes of cB6 mice immunized subcutaneously on the back and two flanks 3 wk previously with 100 μg KLH-DNP in alum. Mechanically disrupted lymph node cell suspensions were

[1] *Abbreviation used in this paper:* DC, dendritic cell.

depleted of low density DC by two rounds of centrifugation on 50% Percoll; the dense fraction was recovered. Part of the resulting population was depleted of B cells by incubation with sheep anti-mouse Dynabeads. On average the resulting populations were <1% F4/80$^+$, 65–74% CD3$^+$, and 24–35% sIg$^+$. After B cell depletion, contamination by B220$^+$ cells was <1%. The transferred population was tested for the presence of Ag-specific T cells by assaying the frequency of IL-3-producing cells in a limiting dilution assay in vitro.

Immunizations. Mice were immunized 1–3 d after reconstitution by injection with 100 μg (or as otherwise indicated) KLH or KLH-DNP or 200 μg OVA in alum precipitate (Serva, Heidelberg, Germany) subcutaneously in the back and two flanks. Animals were killed 7–12 d after immunization, bled by cardiac puncture, and analyzed for T cell responsiveness by in vitro culture.

Tissue Culture Medium and Factors. All cultures were in IMDM supplemented with 2 mM glutamine (Gibco, Paisley, Scotland), 1% penicillin-streptomycin (Gibco), 5 × 10^{-5} M 2-ME (Sigma Chemical Co.), and 10% FCS (Boehringer Mannheim, Mannheim, Germany). Human rIL-2 (Hoffman-La Roche, Nutley, NJ) was obtained through the courtesy of Dr. F. Sinigaglia (Hoffmann-La Roche & Co., Ltd., Basel, Switzerland). Mouse IL-3 was obtained through the courtesy of Dr. G. Le Gros (Ciba-Geigy, Basel, Switzerland).

IL-2 Limiting Dilution Assays. A modification of the method of Rocha and Bandeira (38) was used to determine the frequency of IL-2-producing, Ag-specific cells. Briefly, mouse peripheral lymph nodes and spleens were aseptically removed, mashed by gently forcing through a nylon sieve, and resuspended in medium by pipetting. Graded numbers of total lymph node cell suspensions were plated on 3 × 10^4 cB6 irradiated (2,000 rad) spleen cells plus or minus Ag (OVA, 200 μg/ml; or KLH, 50 μg/ml) in U-bottomed 96-well plates (Costar, Cambridge, MA) in a total volume of 40 μl. Between 21 and 28 wells were plated for each cell concentration; 12 control wells (no responder cells) were set up in each experimental plate. After incubation at 37°C for 36–40 h, the plates were irradiated (2,000 rad), 50 HT-2 IL-2-dependent cells (39) were added to each well in a volume of 10 μl, and incubation was continued for another 48 h. "Rescue" IL-2 (100 μl/well, 100 U/ml) was then added to all wells, and incubation continued for 4 d; labeling with [^{3}H]TdR (5Ci/mmol, 1μCi/well; Amersham Corp., Arlington Heights, IL) was performed in the last 6 h of culture. Individual microcultures were scored as positive or negative based on the incorporations determined on control wells. Regression curves were constructed by plotting the number of responder cells per well versus the log of the percentage of negative wells; only experiments in which regression coefficient (R) > 0.9 were considered. Frequencies were calculated on the regression curve by interpolating the number of responder cells required to give 37% negative cultures (corresponding to one precursor/well according to Poisson statistics). Data analysis using the least square and maximum likelihood methods gave comparable results. Because the percentages of T cells in lymph nodes of different groups of mice varied less than twofold, data are presented as frequencies per total lymph node cells.

IL-3 Limiting Dilution Assay. A modification of the IL-2 limiting dilution assay was used. Cultures were set up in the presence of 10 U rIL-2/ml; after incubation for 72 h at 37°C the plates were irradiated, 80 FD/C.1 IL-3-dependent cells (40) were added to each well in 10 μl volume, and the incubation was continued for 4 d. [^{3}H]TdR pulse was in the last 6 h of the assay.

Determination of the MHC Restriction of Individual Clones. 0.5–2 × 10^6 lymph node cells from KLH-immunized mice were cultured in 24-well plates (Costar) in the presence of 4 × 10^6 irradiated cB6 spleen cells and 50 μg/ml KLH in a total volume of 1.5 ml in order to expand Ag-specific cells. Culture of lymph node cells from nonimmunized mice did not result in detectable cell growth. After 8–10 d of culture, cells were harvested, centrifuged on lympholyte-M (Cedarlane Laboratories, Ontario, Canada) to remove dead cells, and plated at limiting numbers on 3 × 10^4 irradiated cB6 feeder cells with 50 μg/ml KLH and 10 U/ml IL-2 in a total volume of 80 μl in U-bottomed 96-well plates. After 10 d microcultures seeded with optimal numbers of T cells as determined by microscopical examination were centrifuged on a plate carrier, washed twice, and each individual microwell was divided into four aliquots. Each aliquot was plated on 5 × 10^4 BALB/c *nu/nu* or C57BL/6 *nu/nu* irradiated feeder cells in the presence of 10 U/ml IL-2, with or without 50 μg/ml KLH, in a final volume of 50 μl. IL-3 production in individual wells was determined by proliferation of the IL-3-dependent cell line FD/C.1 as described above. Wells where IL-3 production (as measured by FD/C.1 proliferation) in the absence of specific antigen exceeded 20% of the proliferation in the presence of antigen were excluded.

ELISAs. Ag-specific and total Ig responses were determined in a sandwich ELISA by incubating twofold test serum dilutions on ELISA plates (Dynatech) coated with 10 μg OVA-DNP or KLH per well, or 1 μg goat anti-mouse Ig per well (Southern Biotechnology Associates, Birmingham, AL), respectively. The reaction was developed by the addition of 0.2 μg/ml isotype-specific horseradish peroxidase-conjugated goat anti-mouse IgG or IgM (Southern Biotechnology Associates) and 1 mg/ml *O*-phenylenediamine (Sigma Chemical Co.) substrate; 2 M H_2SO_4 was added to stop the reaction. Optical densities were measured on a Titertek Multiskan® MCC/340 ELISA reader (Flow Laboratories AG, Allschwil, Switzerland).

Results

SCID Mice Can Be Reconstituted with Normal T and B Cells. Preliminary experiments were performed in which SCID mice were reconstituted with spleen or lymph node cell suspensions from (BALB/c × B6)F_1 (cB6) mice, and analyzed at different times after injection for homing and persistence of the injected T and B cells. The results demonstrated that donor T and B cells could home to the spleen and lymph nodes of SCID mice and reconstitute a morphologically normal lymphoid structure (B. Scott, F. Ronchese, and M. Kosco, unpublished results). At a population level, injected cells could persist in the host for a significant length of time (3 mo was the maximum time analyzed) without significant alterations in total cell number or in phenotype (CD4/CD8 ratios, percent IgM$^+$ or Ly1$^+$ B cells). These results are in complete agreement with those reported by Sprent et al. (41).

Consistent with the lack of any significant expansion of the number of T cells transferred into SCID mice, no significant increase in the frequency of IL-2-producing, autoreactive T cells could be detected in SCID mice reconstituted with cB6 T or T+B cells 1, 3, or 5 wk before analysis (data not shown). These data indicate that cB6 cells injected into SCID mice do not undergo gross alterations in distribution, number, or reactivity, and can be considered to reflect the peripheral repertoire of a normal mouse. Therefore, SCID mice reconstituted with cB6 T or T+B cells can be used as

a model to study the role of different APC in inducing T cell responses in vivo.

B Cells Are Not Necessary for the Induction of a T Cell Response. SCID mice reconstituted with T or T+B cells were compared for the ability to generate T cell responses after subcutaneous immunization with Ag in alum adjuvant. Nonimmunized spleen suspensions were used as donor cells for transfer into SCID mice as they contain a higher proportion of B cells. T cell responses in the draining lymph node of SCID and control mice were measured by using a limiting dilution assay to assess the frequency of Ag-specific, IL-2-producing cells. This assay was chosen instead of a standard T cell proliferation assay because it was considered to measure a more meaningful parameter of T cell function, such as in vivo clonal expansion and the ability to secrete factors involved in proliferation, help, and activation of effector mechanisms. In addition, it is more sensitive in detecting T cell responses, and can be performed with a low number of cells.

The frequency of IL-2-producing cells in lymph nodes of SCID mice reconstituted with T or T+B cells is comparable to the frequency obtained in similarly immunized cB6 mice; most notably, the frequency of Ag-specific T cells in mice reconstituted only with T cells is as high as the frequency detected in mice reconstituted with T+B cells (Fig. 1). A number of similar experiments performed with mice immunized with KLH or OVA showed comparable results (Table 1); mice not immunized with Ag in vivo failed to generate significant frequencies of IL-2-secreting cells (Table 1). Therefore, DC appear to be sufficient for the induction of Ag-specific, IL-2-producing T cells in vivo; B cells appear not to be necessary.

T cells in the mouse have been functionally divided into Th1, producing IL-2, IL-3, IFN-γ, and LT; and Th2, producing IL-3, IL-4, IL-5, IL-6, and IL-10 (42, 43). It was therefore possible that B cells, although not involved in the priming of IL-2-producing T cells, could nonetheless be required for the priming and/or expansion of Th2 cells. Also, differences between immunization in the presence or in the absence of B cells might be revealed by immunization with limiting doses of Ag. To test these possibilities, reconstituted SCID mice were immunized with lower amounts of Ag and tested for the frequency of Ag-specific, IL-3-producing cells, as this lymphokine is produced by all functional subsets of Th cells (42). Again, no differences could be detected in the frequencies of Ag-specific T cells elicited in the presence or absence of B cells in these conditions (Table 1), or using lower doses of Ag (e.g., 3 μg KLH/mouse; not shown) that still elicited significant responses in both groups. This argues that the failure to detect a difference between priming in the presence or in the absence of B cells was not simply due to the use of "saturating" doses of Ag, or to the exclusion of a subpopulation of T cells dependent on B cells for priming and/or expansion. Also, no in vivo primed T cells should be missed in the in vitro assay used, as it was performed in the presence of high numbers of splenic APC, which are able to sustain proliferation and lymphokine production by both Th1 and Th2 cells (44, 45).

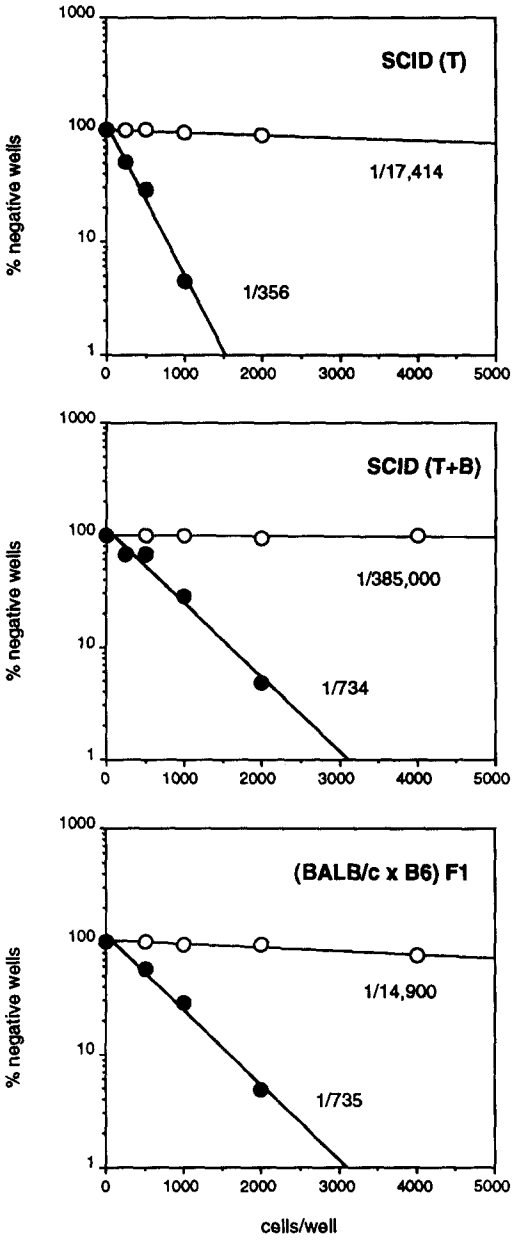


Figure 1. Limiting dilution analysis of the frequencies of KLH-specific IL-2-secreting cells in SCID mice reconstituted with nonimmune splenic T cells or T+B cells from cB6 mice. Reconstituted SCID mice and cB6 mice were immunized subcutaneously with 100 μg KLH in alum and tested for frequency of IL-2-producing cells in the presence (●) or absence (○) of specific antigen 7 d after immunization. Total cell suspensions prepared from the draining lymph nodes were used as effector populations in the limiting dilution assay without any further purification; T cells represented 61% of the total lymph node population in SCID (T) mice, and 50% in SCID (T+B) mice.

Lastly, the possibilities that the transferred B cells were either not functionally active in vivo, or failed to interact with T cells, were also tested. To this purpose, SCID mice reconstituted with T or T+B cells, and immunized with KLH-DNP as above, were bled at the time of death and tested for DNP-specific serum IgM and IgG in an ELISA (Fig. 2). SCID mice that received only T cells failed to generate Ag-specific

Table 1. *Frequencies of Antigen-specific, Lymphokine-producing T Cells in SCID Mice Reconstituted with T or T+B Cells*

Immunizing antigen	Antigen in culture	Lymphokine tested	Experimental group		
			SCID (T)	SCID (T+B)	(BALB/c × B6) F_1
OVA*	OVA	IL-2	2,261‡	3,751	5,968
			8,495	6,673	4,041
			6,213	4,957	
None	OVA	IL-2		51,457	111,657
KLH§	KLH	IL-2	388	333	773
			363	735	1,948
			344	117	
			112	176	
None	KLH	IL-2	47,796	23,391	153,660
KLH‖	KLH	IL-3	1,286	1,175	
			1,520	1,122	

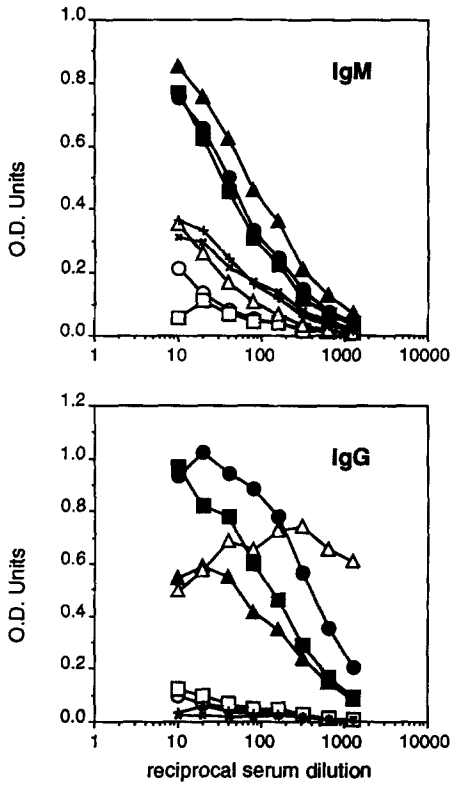
The frequencies of antigen-specific, lymphokine-producing T cells induced by antigen immunization in the presence or in the absence of B cells are comparable. SCID mice reconstituted with nonimmune T or T+B spleen cells from cB6 mice were immunized with antigen in alum adjuvant or left untreated; peripheral lymph node cell suspensions were tested 7–12 d after immunization for frequencies of IL-2- or IL-3-producing cells in a limiting dilution assay. Because the variation in percentages of T cells in lymph nodes from different groups of mice was less than twofold, frequencies per total lymph node population are presented.

* Used at 200 μg/mouse.

‡ Reciprocal of frequency estimates as calculated by linear regression; the frequencies of cells producing lymphokine in the absence of antigen are subtracted.

§ Used at 100 μg/mouse.

‖ Used at 10 μg/mouse.



antibody responses, confirming the lack of functional B cells in these mice. In contrast, mice that received both T and B cells generated significant titers of DNP-specific IgG. No DNP-specific antibodies were detectable in mice immunized with unmodified KLH, demonstrating that the response was in fact Ag specific (Fig. 2). Therefore, B cells in these animals are functional and able to take up Ag and receive T cell help. Thus, failure of B cells to prime T cells cannot be due to their functional incompetence or to inability to interact with Th cells.

B Cells Cannot Prime Naive T Cells. To assess the priming requirements of naive T cells, cB6 single-positive thymic T cells were transferred into SCID mice in the presence or absence of cB6 splenic B cells. Thymic T cells were chosen be-

Figure 2. Production of specific antibodies in SCID mice reconstituted with nonimmune splenic T or T+B cells. Reconstituted SCID mice and cB6 mice were immunized subcutaneously with 100 μg KLH-DNP and tested for DNP-specific serum IgM and IgG in an ELISA. (□ and ○) SCID mice reconstituted with T cells only and immunized with KLH-DNP 9 d previously; (■ and ●) SCID mice reconstituted with T+B cells and immunized with KLH-DNP 9 d previously; (×) SCID mouse reconstituted with T+B cells and immunized with nonmodified KLH 9 d previously; (▲) cB6 mouse immunized with KLH-DNP 7 d previously; (△) cB6 mouse immunized with KLH-DNP 35 and 7 d previously; (+) cB6 normal serum.

cause of the lack of suitable surface markers to isolate naive T cells from the peripheral repertoire of normal mice. Single-positive cells were separated from the bulk of the immature thymocyte cell populations by negative selection with the J11d mAb (34, 46). When transferred into SCID mice these cells behaved in a fashion similar to T cells from peripheral organs in terms of homing and survival in the host. SCID mice reconstituted with T or T+B cells were immunized with KLH-DNP in alum and killed 9–12 d after immunization. Lymph node cell suspensions were cultured on cB6 feeder cells in the presence of specific Ag and subsequently assayed for MHC restriction of the responding T cells as described in Material and Methods.

Fig. 3 shows the results of one such experiment in which the Ag-specific activity of individual wells is compared for T cells from control cB6 mice or from SCID mice reconstituted with T or T+B cells. Ag-specific T cells from cB6 mice (H-2^d × H-2^b) fell into two groups: T cells that recognized Ag in the context of H-2^d (i.e., able to recognize Ag on BALB/c but not B6 APC), and H-2^b-restricted T cells that showed the reciprocal behavior, i.e., reactivity to Ag plus B6 and not Ag plus BALB/c APC. This implies that H-2^d and H-2^b are equally able to present KLH antigen to T cells. Nonetheless, when T cells from SCID mice (H-2^d) reconstituted with cB6 (H-2^d × H-2^b) T or T+B cells were analyzed in this assay, all the Ag-specific activity was restricted to H-2^d-expressing BALB/c APC regardless of the presence or absence in the host of B cells expressing H-2^b gene products. Transfer of B cells from mice previously primed to DNP, and therefore presumably enriched in DNP-specific B cells (Fig. 3), or of B cells from nonimmune mice, did not affect the result of the experiment. It was possible that the transferred B cells might not be functional or able to interact with T cells; however, expression of increased levels of class II and production of Ag-specific IgG in these mice (not shown) demonstrated that this was not the case.

Therefore, naive T cells can only be primed by Ag presented on DC, regardless of the presence in vivo of Ag-specific, activated B cells.

B Cells Can Act as APC to Recall Responses by Previously Primed T Cells. Naive and Ag-experienced T cells have been reported to express different levels of adhesion molecules (3) and to show different activation requirements in vitro (47–49); therefore, they may also show different requirements in terms

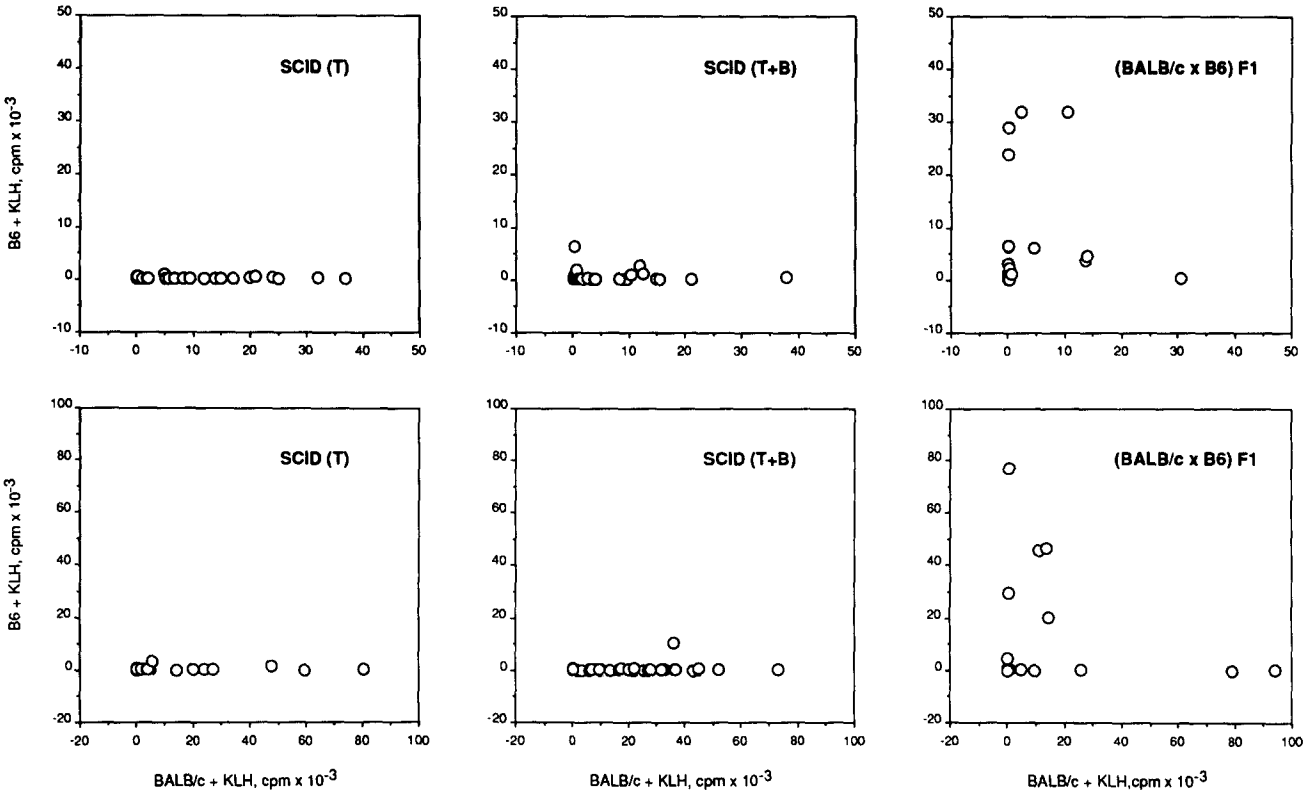


Figure 3. Analysis of the MHC restriction of antigen-specific T cells recovered from immunized SCID mice reconstituted with naive (single-positive thymic) T cells or with naive T and splenic B cells from DNP-primed mice. Each panel corresponds to one mouse; each dot represents antigen-specific IL-3 production of a single microculture as measured by proliferation of an IL-3-dependent cell line. Reconstituted SCID mice and cB6 mice were immunized subcutaneously with 100 μg KLH-DNP in alum; cells from the draining lymph nodes were cultured on irradiated cB6 feeder cells and KLH, and plated into 96-well plates as detailed in Material and Methods. Individual microwells were subsequently assayed for MHC restriction by dividing into four aliquots and testing each aliquot on BALB/c or B6 feeder cells in the presence or absence of KLH; results obtained with two different cell inputs/well are pooled in each panel. The percentage of wells giving a response varied between 3 and 60%, and was usually between 10 and 40%. Microcultures responding to both parental haplotypes are most likely due to the simultaneous presence of more than one precursor in the same well.

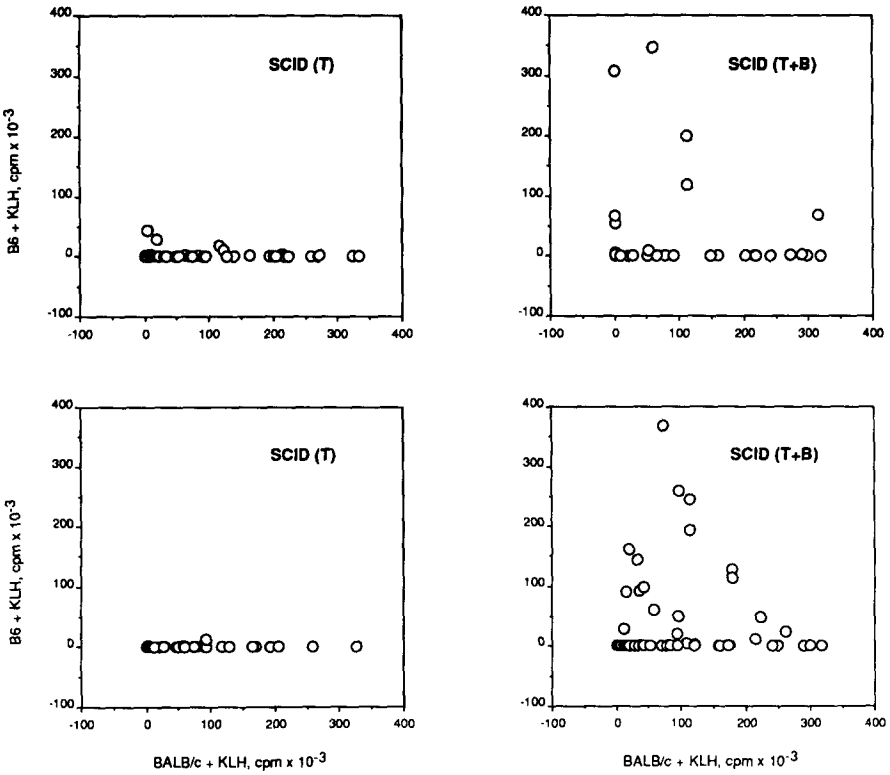


Figure 4. Analysis of the MHC restriction of antigen-specific T cells recovered from immunized SCID mice reconstituted with KLH-DNP-specific "antigen-experienced" T cells or with KLH-DNP-specific T and B cells. Each panel corresponds to one mouse; each dot represents antigen-specific IL-3 production of a single microculture as measured by proliferation of an IL-3-dependent cell line. Reconstituted SCID mice were immunized subcutaneously with 100 μg KLH-DNP on alum; cells from the draining lymph nodes were cultured on irradiated cB6 feeder cells and KLH, and plated into 96-well plates as detailed in Material and Methods. Individual microwells were subsequently assayed for MHC restriction by dividing into four aliquots and testing each aliquot on BALB/c or B6 feeder cells in the presence or absence of KLH; results obtained with two different cell inputs/well are pooled in each panel. The percentage of wells giving a response varied between 15 and 85%, and was usually between 30 and 60%. Microcultures responding to both parental haplotypes are most likely due to the simultaneous presence of more than one precursor in the same well.

of Ag presentation in vivo. To assess whether this is the case, Ag-primed T cells were used to reconstitute SCID mice. cB6 mice that had been immunized subcutaneously with KLH-DNP 3 wk previously were used as a source of Ag-primed T cells; such T cells are restricted to both the MHC haplotypes present in the donor strain, H-2^d and H-2^b. SCID mice were reconstituted with lymph node cell suspensions that were depleted or not depleted of B cells, and were again immunized subcutaneously with KLH-DNP; the restriction of Ag-specific T cells recovered from the draining lymph nodes was then analyzed.

As shown in Fig. 4, SCID mice (H-2^d) that received purified T cells from immune cB6 mice showed a response exclusively H-2^d restricted; the H-2^b-restricted T cell subpopulation was most likely not expanded or not recalled to effector phenotype due to the absence in the host of APC expressing the appropriate MHC. In contrast, H-2^b-restricted T cells were readily demonstrated in SCID mice that had received both T and B cells from immunized cB6 mice, despite the inability of the assay to detect I-E^b-restricted T cells.

Since in these reconstituted SCID mice H-2^b was present only on B cells and not on macrophages or DC, we conclude that, unlike naive T cells, Ag-experienced T cells are able to be restimulated by Ag presented on B cells.

Discussion

The present study was aimed at defining the role of B cells versus other APC in the presentation of Ag to class II–restricted T cells in vivo. Two specific questions were asked: are B cells required for priming of T cells, and are B cells able to present Ag directly to T cells in primary and/or secondary immune responses?

To analyze the requirement of B cells for the induction of a T cell response, a limiting dilution assay for IL-2- and IL-3-producing cells was used to evaluate the clonal expansion of Ag-specific T cells induced by immunization in the presence or absence of B cells. The results clearly showed that B cells or their products are not required for the initiation of a T cell immune response; presentation of Ag to class II–restricted T cells in vivo is carried out preferentially by other APC. The precise identity of such a "professional" APC was not further investigated in this study, and the results are equally compatible with it being a DC or a macrophage. However, numerous studies suggest that DC, and not macrophages, are the main APC population for the priming of class II–restricted T cells in vivo (50, 51) and in vitro (52, 53).

The outcome of the experimental protocol described here could have been influenced by the choice of immunization procedure, e.g., soluble vs. particulate Ag, and by the assay chosen to test T cell function. For example, particulate Ag (such as the alum precipitate used here) may not be taken up efficiently by nonphagocytic cells such as B cells, while soluble Ag might. Also, specific T cell subpopulations might respond suboptimally to Ag presented on B cells; analysis of such T cell subpopulations would, of course, fail to reveal differences between priming in the presence or in the absence of B cells. Neither of these possibilities appears to apply to the present study. Production of specific antibodies of the IgG isotype suggests that B cells had been exposed to Ag

adequately, and were able to interact with T cells to receive cognate help. This implies B cells were sufficiently activated (even in the absence of bacterial components in the adjuvant) to be able to act as effective APC for T cells. Also, although a complete characterization of the lymphokines produced by T cells was not attempted, analysis of two different lymphokines, IL-2 and IL-3, produced by partially overlapping subsets of T cells (42), failed to reveal differences related to the presence or absence of B cells. Therefore, the finding that B cells are not required for the induction of a primary T cell response does not appear to be simply a consequence of the experimental system used, but might also apply to the presentation of other types of Ag, entering the organism via different routes.

Previous reports have used mice depleted of B cells by chronic anti-μ treatment (12–14, 16), or lacking expression of a specific class II molecule on B cells (54), to show that B cells are required for the generation of lymph node proliferative T cell responses after in vivo Ag immunization. It is not clear why no or suboptimal T cell responses were detected in such studies, as opposed to the normal responses reported here. One possibility is that, as different readout systems were used, B cells might be required for the generation of proliferative but not lymphokine responses. Other authors (55) have, however, reported that SCID mice reconstituted with syngeneic thymocytes could generate normal proliferative and IL-2 responses after in vivo immunization in the absence of B cells; preliminary results showed that also in our case T cells from SCID mice reconstituted only with T cells were able to proliferate in vitro, and could generate long-term Ag-specific T cell lines. Residual B cell contamination is also probably not responsible for the ability of reconstituted SCID mice to generate T cell responses. By FACS® staining, contamination by donor or host type B cells in the spleen or lymph nodes of SCID mice reconstituted only with T cells was below detection, therefore at a level lower or comparable to the level detected in anti-μ-treated mice (12, 14). The very low levels of serum Ig detected in SCID mice reconstituted with T cells only (and in noninjected SCIDs), mostly of the μ isotype and that failed to show Ag-specific activity, could possibly be due to leaky B cells, described to occur in the peritoneal cavity of older SCID mice (56). Due to their distribution these cells are, however, unlikely to contribute to responses taking place in the lymph node. Lastly, it is possible that Ag presented by DC is limiting in anti-μ but not in SCID mice. The low number of T cells present in SCID mice would allow any T cell access to Ag presented on DC; the same might not be the case in anti-μ mice, where the ratio of T cells to DC is much higher. Expansion of specific T cells on Ag-loaded B cells would then be required for detection of a response in anti-μ mice, but not in SCIDs. However, the low number of Ag-specific B cells that are able to take up and present efficiently to T cells, as compared with the much larger number of DC, makes this possibility rather unlikely. Further experiments making use of different experimental models (57, 58) should help to resolve this issue.

The experiments discussed so far establish that B lymphocytes are not required for the initiation of a primary T cell response in vivo; however, they do not exclude that B cells might be able to present directly to T cells when in the absence of the dominant APC population, DC. To test this possibility, the MHC restriction of Ag-specific T cells in the SCID chimeras was determined. B cells in these mice were the only cells bearing I-A^b; therefore, any I-A^b-restricted, Ag-specific T cells could have been primed only on B cells. The results obtained showed quite clearly that naive T cells were primed by Ag presented on DC but not on B cells; however, B cells were able to reactivate previously primed T cells. The difference in the ability of B cells to elicit secondary but not primary Ag-specific T cell responses in vivo was not due to a different degree of reconstitution of the B cell compartment in the two groups of mice, as the ratio of B vs. T cells was actually higher in mice reconstituted with naive rather than primed T cells; nor was it related to the presence of Ag-specific B cells, as naive T cells could not be primed by B cells even when the B cells were from Ag-primed donors. Production of specific antibodies showed that B cells had been loaded with Ag and were able to interact productively with T cells. Also, H-2^b-restricted responses could be demonstrated in SCID mice reconstituted with naive T cells and immunized by injection of Ag-pulsed DC from cB6 (H-2^d × H-2^b) donors (not shown), showing that H-2^b is not intrinsically worse than H-2^d in presenting Ag, and that T cells were able to use the I-A^b restriction element if the appropriate APC were present. Therefore, we interpret our results as indicating an absolute incapacity of B cells to stimulate primary Ag-specific T cell responses in vivo. Results consistent with these findings have also been reported using in vivo systems by Sprent (59) in the mouse and Lassila et al. (17) in the chicken; and in vitro by Inaba and Steinman (52, 53). In all cases B cells were shown to be inefficient in stimulating resting T cells, but able to receive help by already activated T cells.

Unlike resting B cells, activated B cells have been reported to be very efficient APC in vitro (60, 61), and express adhesion molecules that are absent in resting B cells (62). If, however, B cells require a T cell–derived signal to be activated and become efficient APC (63, 64), failure to induce specific T cells could in turn prevent the activation of B cells into APC potentially able to prime other T cells. The experimental design used in this study allowed us to test this possibility. After activation by H-2^d-restricted T cells, B cells in SCID mice expressed high levels of class II (not shown) and produced antibodies. Once activated they should theoretically have been able to prime T cells, such as H-2^b-restricted ones. As no H-2^b-restricted T cells could be demonstrated, we conclude that B cells, either resting or activated, are unable to prime naive T cells. Most likely, the inability of B cells to present Ag to naive T cells is due to their lack of specific adhesion molecules (3, 65). Memory T cells are reported to express higher levels of adhesion molecules than naive T cells (3); such high levels render them more easily triggered when exposed to APC expressing the correct Ag ligand. In a reciprocal fashion, expression of higher levels of adhesion molecules, or of additional ones, could render DC more efficient APC than B cells. B7/CD28 is one adhesion molecule/ligand

pair whose importance in APC/T cell interactions has recently been recognized (66): simultaneous engagement of TCR and CD28 leads to increased T cell proliferation and lymphokine production (67, 68), and can override tolerance induction by APC that lack a costimulatory signal (69, 70). The ligand for CD28, BB1/B7, is expressed on DC (71), IFN-γ-activated macrophages (72), and on activated but not resting B cells (62); its expression may transiently follow T-B cell cognate interaction (73). If coengagement of CD28 and TCR were the only signals required for priming naive T cells, activated B cells would be expected to prime T cells. Our results suggest that this is not the case and that B cells, either resting or activated, are truly unable to prime T cells. Our results do not address the possibility that B cells, if unable to prime, would rather deliver a negative signal resulting in the functional inactivation of naive T cells. Another more trivial possibility is that the compartmentalization of cells in lymphoid organs may favor naive T cells encountering Ag on DC but not on B cells. Only after T cells have been stimulated by Ag on APC in the T cell area of the lymph node would they migrate to the B cell areas to deliver help through recognition of Ag presented on B cells. If this is the case, it could represent (under physiological conditions of Ag distribution and presentation) a way to prevent encounter of naive T and B cells that, as recently reported, could induce tolerance in naive T cells (74).

Previous studies have reported that activated but not resting B cells are able to prime normal T cells to alloresponses in vitro (75, 76). Since peripheral T cells were used as a source of responding T cells, and since such T cell preparations comprise not only naive T cells but also a significant proportion of Ag-experienced T cells, it is quite likely that the T cell responses elicited by B cells were, like in the present study, due to Ag-experienced T cells. This also predicts that any T cell response in an adult animal, where a proportion of the peripheral T cell pool is Ag experienced, would include recall of crossreactive memory T cells, and may involve Ag presentation by B cells. Evidence that such a mechanism may indeed take place in vivo was obtained in experiments in which SCID mice were reconstituted with high density splenic T+B cells from nonimmunized mice, and found to be able to generate low numbers of H-2^b-restricted T cells after Ag immunization (not shown). An analogous mechanism could explain a recent study where induction of lymph node proliferative responses was reported to follow the induction of activated B cells in vivo (77): suboptimal priming on DC would only be revealed after expansion on Ag-specific B cells.

The results reported in this paper do not allow us to discriminate whether Ag presented by B cells to previously activated T cells either induces proliferation and clonal expansion of such T cells, or T cells are "recalled" to an effector phase in the absence of any proliferation, or are simply rescued from being eliminated. Whatever the mechanism, the ability of B cells to elicit secondary T cell responses may constitute a mechanism by which T cell memory is maintained. Previous studies have suggested that B cell memory could be maintained by the continuous exposure to Ag (78) stored on follicular dendritic cells in lymph node follicles (79, 80). B cells, which were shown to be able to take up, internalize, and present Ag stored on follicular dendritic cells (81), could therefore act as APC for the maintenance of memory T cells.

Our results show that B cells are unable to prime naive T cells in vivo, but are capable of eliciting Ag-specific responses in previously primed T cells. This latter property of B cells might be especially important in adult animals in which, due to thymus involution, a relevant fraction of the peripheral T cell pool is constituted by Ag-experienced cells. This finding may lend some support to the hypothesis that selective in vivo activation of specific T cell subpopulations, producing distinct patterns of lymphokines, correlates with the type of APC participating in the response in vivo (45, 82, 83). T cells receiving signals through the engagement of different sets of adhesion molecules, whose ligands are differentially expressed by DC and by B cells, would be driven to production of different lymphokines, thus marking the response as Th1 or Th2. This could possibly constitute a mechanism for the regulation of the immune response.

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