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Targeting of T–B interaction using heteroconjugate antibody

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SUMMARY

We have deliberately targeted collaboration between T cells and certain B cells by using a heteroconjugate (HETCONJ) antibody. This specific reagent was created by cross-linking the $F(ab')_2$ portions of anti-I-A^b and anti-CD3 monoclonal antibodies. Spleen cells from B6 (I-A^b) but not bm12 (I-A^{bm12}) mice proliferated *in vitro* in the presence of the HETCONJ. Similarly, T-cell dependent IgM secretion was induced in B cells from B6, yet only weakly in B cells from bm12 mice. Using B cells from Igh allotype double congenic (B6.C20 Igh^a/I-A^b and bm12, Igh^b/I-A^{bm12}) mice in co-culture experiments, we have used the HETCONJ to study linked versus bystander T–B interaction. B-cell activation, mediated by HETCONJ, was 10 times greater in unseparated than in resting splenic B cells. T–B interaction through T–B contact was more efficient than activation through bystander effects both for unseparated and resting splenic B cells. Large, already activated B cells, in contrast, did not show a preference for linked recognition. Our reagent has mimicked some of the events involved in T–B collaboration and may be useful in studying the molecular basis of cellular interactions.

INTRODUCTION

B-cell responses against thymus-dependent antigens (Ag) require specific T-helper cell activation and release of factors.[1,2] This complex interaction involves processing of the Ag which is then presented to T cells in conjunction with Ia molecules.[3] *In vitro* and *in vivo* studies have compared the requirements for cognate (through specific recognition)[4–6] and bystander (non-specific, through lymphokines)[7] B-cell activation. In specific T–B interaction, a trimolecular complex composed of T-cell receptor (TcR)–Ag–Ia is formed[8] and contributes to T–B conjugation. In addition, adhesion molecules appear to stabilize this conjugate.[9,10] In non-specific T–B interaction, B cells are thought to be activated by T-cell factors without the involvement of a specific TcR–Ag–Ia complex. In that case, however, the role of adhesion molecules is not excluded. To study and control these interactions, artificial ways of mimicking them have been devised. The original one described linked recognition through the carrier–hapten effect.[11] More recently, cross-linking of anti-myoglobin or anti-ferritin antibodies to anti-mouse Ig,[12,13] or anti-major histocompatibility complex (MHC), or anti-Ig to anti-DNP[14] have been shown to enhance B-cell presentation of specific antigens and induce *in vivo* responses to weak antigens. Anti-Thy-1–TNP conjugates were also used to show the importance of T-cell activation via the TcR in T–B co-operation.[15]

In this paper, we have mimicked some of the events involved in T–B co-operation by broadly targeting two of the molecules specifically involved in this interaction: CD3 on T cells and I-A on B cells. Anti-CD3 antibodies have been shown to induce T-cell activation.[16] Ia molecules have been implicated in trans-membrane signalling to B cells.[17] The $F(ab')_2$ fragments of these monoclonal antibodies (mAb) were coupled to each other to give a bispecific, heteroconjugate (HETCONJ) reagent. We used mice doubly congenic for I-A (I-A^b and I-A^{bm12}) and IgM allotype (Igh^a and Igh^b) to compare linked and bystander T–B interaction in co-culture experiments.

MATERIALS AND METHODS

Mice

(Table 1) I-A co-isogenic C57BL/6Kh (B6, I-A^b, Igh^b) and B6.C-H-2 bm12 (bm12, I-A^{bm12}, Igh^b) mice were originally obtained from Dr R. Melvold (Northwestern University, Chicago, IL) and maintained in our colony. C57BL/6.C-20 (B6.C20, I-A^b, Igh^a) mice were obtained from Dr G. Bosma (Institute for Cancer Research, Philadelphia, PA). (B6.C20 × bm12)F_1 mice were produced in our colony. Three- to six-month-old mice of both sexes were used.

Antibodies

Rat IgM anti-Thy-1.2 (AT83),[18] anti-CD8,[19] and anti-CD4[20] mAb were used for complement-mediated cytotoxicity. Hams-

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Abbrevation: HETCONJ, anti-CD3/anti-IA^b heteroconjugate.

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Table 1. MHC haplotypes and Ig allotypes of mice used

Mouse strain	Class II	Igh
B6.C20	$I\text{-}A^b$	a
bm12	$I\text{-}A^{bm12}$	b
(B6.C20 × bm12) F_1	$I\text{-}A^b/I\text{-}A^{bm12}$	a or b

ter IgG anti-murine CD3 mAb (145-2C11 = 2C11, a gift from Dr J. Bluestone, University of Chicago, Chicago, IL)[21] was purified as described elsewhere[22] from serum-free supernatant (grown in HB 102 medium; Hana Biologics, Alameda, CA) over HPLC (Millipore, Waters, Milford, MA) ion-exchange columns. Murine IgG2a anti-$I\text{-}A^b$ mAb (D3-137.5), a gift from Dr S. Tonkonogy (North Carolina State University, Raleigh, NC), was purified from ascites by precipitation with ammonium sulphate, followed by protein A column (Sigma, St Louis, MO) separation. Anti-allotype mAb HB100 (anti-IgM^a, rat IgG1)[23] and AF6-78.25 (anti-IgM^b, mouse IgG1)[24] were obtained, respectively, from American Type Culture Collection, Rockville, MD and from Dr A. M. Stall (Stanford Medical School, Stanford, CA).

$F(ab')_2$ heteroaggregate preparation

$F(ab')_2$ fragments were prepared by 1% pepsin digestion at 37° of mAb in 0·5 M sodium acetate (pH 4·5) for 16 hr. They were then separated over a Sephadex G 200 (Pharmacia, Piscataway, NJ) column followed by further purification with a protein A column (for 145-2C11) or an anti-mouse pFc′ column (for D3-137.5). The conjugation of $F(ab')_2$ fragments of the two mAb was performed as described elsewhere.[25]. Briefly, $F(ab')_2$ fragments in phosphate buffer (pH 7·5) were incubated separately for 2 hr at room temperature in the presence of an 8 molar excess of N-succinimidyl 3-[2-pyridyldithio]-propionate (SPDP); Sigma). 145-2C11 $F(ab')_2$ were then treated with dithiothreitol (0·02 M; Sigma) and added to D3-137.5 $F(ab')_2$. After 9 hr of incubation at room temperature, 300 mg of iodoacetamide (Sigma) were added. The heteroconjugates were separated over a Sephadex G 200 column, sterilized by filtration, and kept at 4°.

Preparation of cells

Mice were killed by cervical dislocation and spleens were removed aseptically. Single-cell suspensions were prepared in Dulbecco's modified Eagle medium (DMEM; Hazelton Research Products, Denver, PA) supplemented with 10 mM HEPES. B cells from B6.C20 and bm12 mice were prepared from spleen cells by complement-mediated cytotoxicity using a mixture of anti-Thy-1.2, anti-CD8 and anti-CD4 mAb. Spleen cells from (B6.C20 × bm12) F_1 mice were enriched for $CD4^+$ cells with a panning technique using Petri dishes coated with a previously determined optimal concentration of goat anti-mouse IgM antiserum followed by treatment with anti-CD8 + complement. Small and large B cells were prepared by separation of purified B cells over a Percoll gradient, as described elsewhere.[26] Small B cells were taken at the 60–70% interface and large B cells at the 50–60% interface.

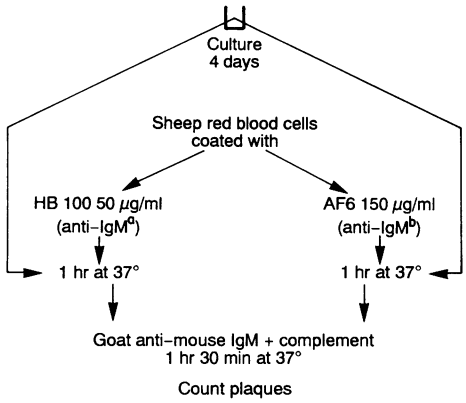

Figure 1. Schematic description of the allotype-specific plaque assay.

Cell cultures

All cultures were performed in DMEM supplemented with non-essential amino acids, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, 100 μg/ml streptomycin, 100 U/ml penicillin and 10% foetal calf serum (FCS; Hazelton). Proliferation assays were performed in 96-well round-bottomed microtitre plates (Corning, Houston, TX). Cells (10^5 cells/well) in 200 μl were cultured in the presence of various doses of mAb or heteroconjugate at 37° in an incubator containing 5% CO_2 in humidified air. One μCi of [^{3}H]thymidine ([^{3}H]TdR; ICN, Irvine, CA) was added to each well after 40 hr of culture and incubated for 8 hr before harvest with an automated device (PhD harvester, Cambridge Technology, Cambridge, MA). One-millilitre cultures were performed in 24-well plates (Costar, Cambridge, MA). B6.C20 and bm12 B cells were co-cultured at $0{\cdot}8 \times 10^6$ cells/ml in the presence or absence of 5×10^4 (B6.C20 × bm12)F_1 $CD4^+$-enriched T cells for 4 days. Unconjugated mAb and heteroconjugates were added at the indicated concentrations. As controls, B cells from B6.C20 or bm12 mice were cultured separately with heteroconjugate or with lipopolysaccharide (LPS) 055:B5 (Difco, Detroit, MI) (50 μg/ml).

Allotype-specific IgM plaque-forming cell (PFC) assay

(Figure 1) Antibodies were coupled to sheep red blood cells (SRBC). Equal volumes of packed SRBC, chromic chloride (Mallinkrodt, St Louis, MO) (0·5 mg/ml), and antibody (50 μg/ml of HB100 or 100 μg/ml of AF6-78.25) were incubated at room temperature for 25 min in normal saline. After three washes in saline, the antibody-coupled SRBC were diluted 1/10 in Hanks' balanced salt solution (Tissue Culture Facility, Lineberger Cancer Center, Chapel Hill, NC) containing 100 μg/ml streptomycin, 100 U/ml penicillin, 10 mM HEPES, and 0·075% $NaHCO_3$ (HBSS). The coupling efficiency was evaluated in an agglutination assay using anti-mouse IgG1 and anti-rat IgG1 antisera. The PFC assay was performed as described elsewhere.[27] Briefly, cells were harvested from culture wells, washed three times, and suspended in 1 ml of HBSS. Cells (100 μl/well) were distributed in triplicate in flat-bottomed microtitre plates (Corning, Houston, TX). Ten microliters of a 10% solution of SRBC coupled either with HB100 or with AF6-78.25 were then added, and the plates were centrifuged and allowed to incubate for 1 hr at 37° in 5% CO_2 in air. Goat anti-mouse IgM antiserum (10 μl/well, final dilution 1/100) and guinea-pig complement (Pel-Freez, Rogers, AR) (10 μl/well, final dilution

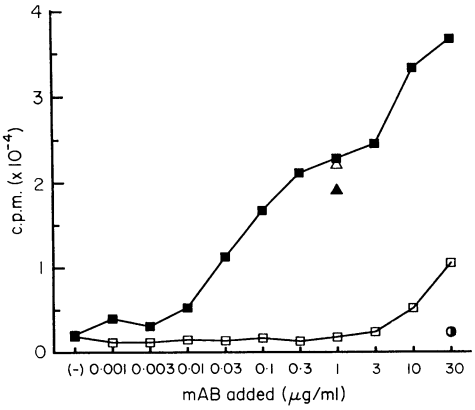

Figure 2. Induction of B6 spleen cell proliferation by the HETCONJ. Spleen cells (10^5/well) from B6 (filled symbols) or bm12 mice (empty symbols) were cultured in triplicate in the presence of 1 μg/ml of anti-CD3 (2C11), 30 μg/ml of $F(ab')_2$ fragments of 2C11 and anti-I-A^b (D3-137.5), or varying doses of the anti-CD3/anti-I-A^b HETCONJ. [■] B6+HETCONJ; (□) bm12+HETCONJ; (▲) B6+2C11; (△) bm12+2C11; (●) B6+$F(ab')_2$ anti-CD3+$F(ab')_2$ anti-1-A^b; (○) bm12+$F(ab')_2$ anti-CD3+$F(ab')_2$ anti-I-A^b.

1/13) were added together to each well and the plates further incubated at 37° for 90 min. PFC were counted using a dark field stereo microscope.

RESULTS

2C11/D3-137.5 heteroconjugate (HETCONJ)-specific proliferation of B6 spleen cells

The bispecific HETCONJ was first tested for its capacity to elicit spleen cell proliferation. B6 and bm12 spleen cells were incubated separately in the presence of various dilutions of HETCONJ. IgG (whole molecule) anti-CD3 antibody was used as a positive control. As shown in Fig. 2, both B6 and bm12 spleen cells responded well to anti-CD3. However, the HETCONJ induced efficient proliferation of only B6 spleen cells, demonstrating the specificity of this reagent. A mixture of $F(ab')_2$ fragments of anti-CD3 and anti-I-A^b (30 μg/ml) had no effect either on B6 or on bm12 spleen cells.

Specificity of PFC assay for IgM^a and IgM^b

LPS-stimulated B cells were tested to demonstrate the specificity of the allotype PFC assay. As shown in Fig. 3a, only IgM^a allotype PFC were detected when B6.C20 B cells were cultured with LPS. Conversely, only IgM^b allotype PFC were detected with bm12 B cells cultured with LPS. Moreover, similar numbers of PFC were obtained with B6.C20 and bm12 B cells, implying comparable sensitivity in the detection of either allotype. When B6C.20 and bm12 B cells were co-cultured, similar numbers of PFC of both allotypes were detected. These results established that PFC of both allotypes could be discriminated and that co-culture of IgM^a with IgM^b B cells did not influence the number of PFC specific for each allotype. As expected, LPS-stimulated F_1 B cells produced similar amounts of IgM^a and IgM^b PFC.

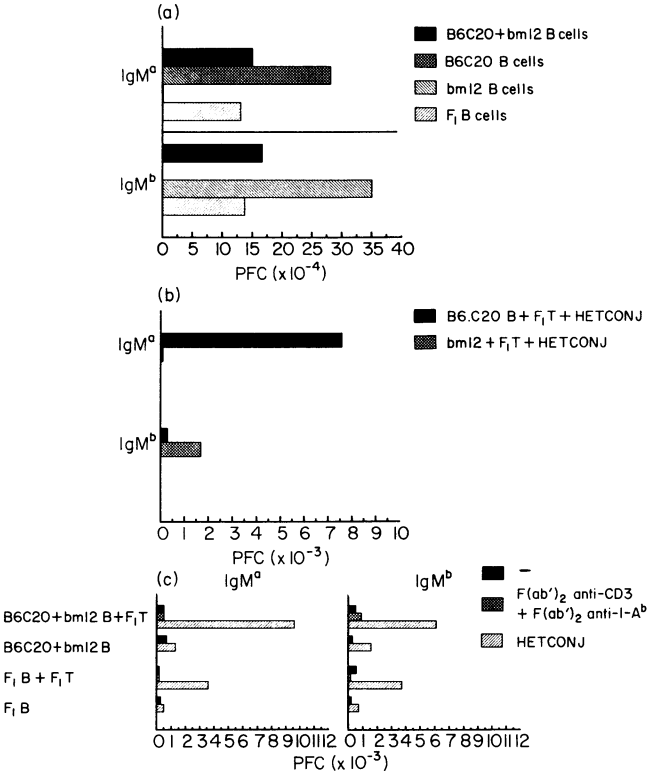

Figure 3. Linked and bystander T–B interaction in splenic B cells. (a) Allotype plaque assay specificity. $0{\cdot}5 \times 10^6$ B cells from B6.C20, bm12 or (B6.C20 × bm12)F_1 mice were co-cultured or cultured separately for 4 days in the presence of 50 μg/ml of LPS. The allotype-specific plaque assay was then performed in triplicate. (b) HETCONJ specificity. B cells from B6.C20 and bm12 mice (10^6/well) were cultured separately in the presence of 5×10^4 F1 $CD4^+$-enriched T cells and the HETCONJ (3 μg/ml). (c) Linked versus bystander effect. Mixtures of B6.C20 and bm12 B cells were co-cultured as described in (b) in medium alone, in the presence of a mixture of $F(ab')_2$ antibodies (1·5 μg/ml), or the HETCONJ (3 μg/ml). As a control (B6.C20 × bm12)F_1 B cells were cultured in the same conditions. After 4 days, an allotype-specific plaque assay was performed. The results are expressed as PFC/10^6 B cells. One standard deviation did not exceed 10% when responses were above background.

Targeting of B-cell activation with 2C11/D3-137.5 HETCONJ

Figure 3b shows typical results obtained when B6.C20 and bm12 B cells were cultured separately in the presence of HETCONJ. In the presence of (B6.C20 × bm12)F_1 $CD4^+$-enriched T cells, B6.C20 B cells exhibited a substantial activation as determined by the large numbers of IgM^a PFC. As would be predicted, no IgM^b PFC were seen. On the other hand, bm12 B cells, bearing Ia molecules which are non-reactive with the HETCONJ, showed little IgM^b secretion and no IgM^a secretion.

Linked versus bystander effect in HETCONJ stimulation

To determine the importance of linked versus bystander effects in HETCONJ-mediated T–B interaction, we co-cultured B6.C20 and bm12 B cells in the presence of (B6.C20 × bm12)F_1 $CD4^+$ cells either in medium alone, with a mixture of $F(ab')_2$ antibodies, or with HETCONJ. As a control,

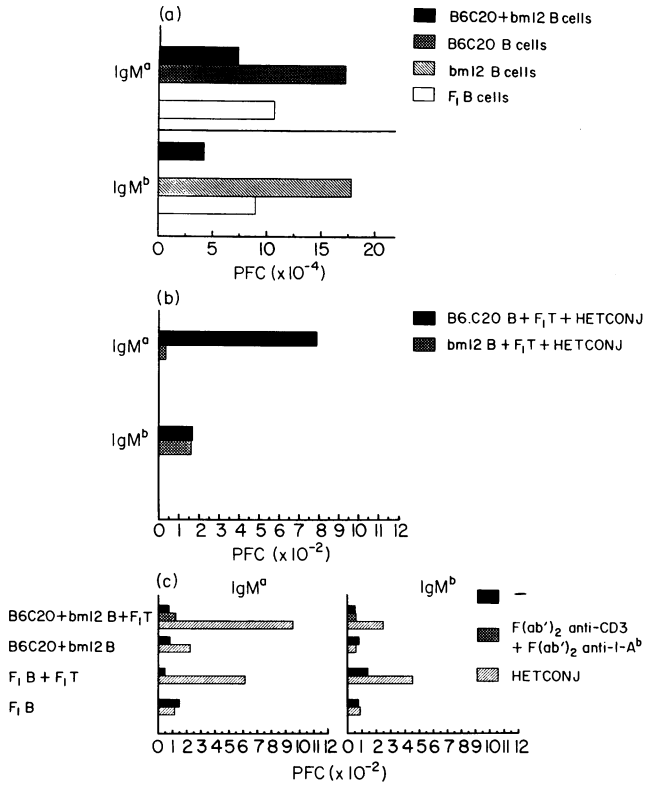

Figure 4. Linked and bystander T–B interaction in small B cells. See Fig. 3 legend. T-depleted spleen cells were separated over a Percoll gradient into small and large B cells. Co-culture experiments as described in Fig. 3 were performed using 10^6 B cells and 2×10^4 $CD4^+$-enriched F_1 T cells. HETCONJ was used at 0·3 μg/ml and the $F(ab')_2$ fragments at 0·15 μg/ml. An allotype plaque assay was performed after 4 days of culture.

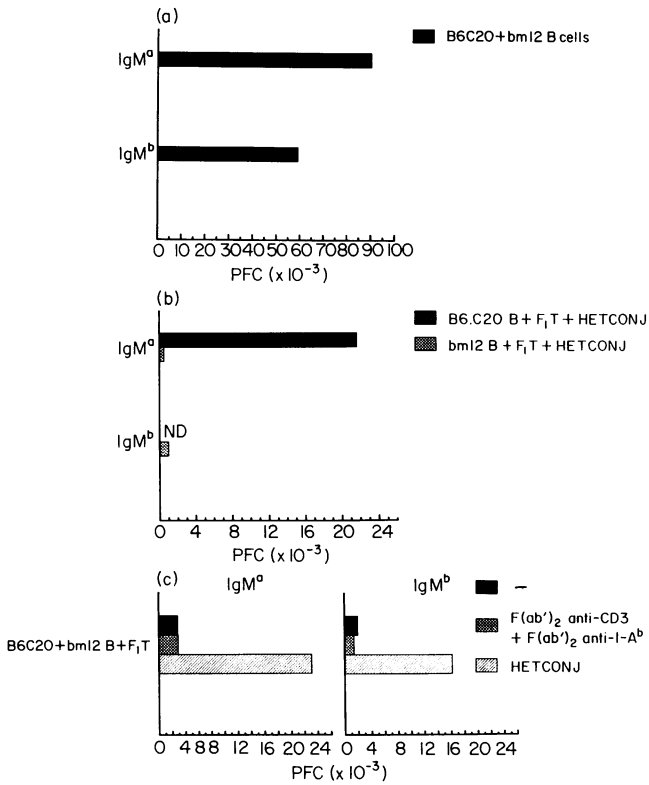

Figure 5. Linked and bystander T–B interaction in large B cells. See Fig. 4 legend.

(B6.C20 × bm12)F_1 B cells were cultured in the same conditions. As shown in Fig. 3c, few plaques were detected after culture with a mixture of $F(ab')_2$ fragments of the anti-CD3 and anti-I-A^b mAb. Furthermore, in the absence of T cells, little B-cell IgM secretion was seen. In the presence of F_1 T cells and HETCONJ, however, more IgM^a than IgM^b PFC (9600 versus 6100) were observed, indicating a preference for cognate interaction, although substantial bystander effect was observed. F_1 B cells, in contrast, produced an equal number of IgM^a and IgM^b PFC. Similar results were obtained in a total of four experiments, and the mean (±1 SD) ratio of HETCONJ/T cell-induced B6.C20 (linked) PFC to bm12 (bystander) PFC was 1·94 (±0·31).

Linked versus bystander effect on small and large B cells with HETCONJ stimulation

Unseparated splenic B cells represent a mixture of activated and resting B cells. To determine the relative dependence on linked T–B interaction of the two populations, we separated them over a Percoll gradient and cultured them under various conditions. Control stimulation with LPS (Fig. 4a) showed some excess of IgM^a over IgM^b in B6.C20 + bm12 co-cultures. However, when B6.C20 or bm12 B cells were cultured separately in the presence of LPS, they produced equivalent amounts of their respective IgM, while F_1 B cells also generated similar amounts of IgM^a and IgM^b PFC (Fig. 4a). The HETCONJ was able to induce activation of B6.C20 small B cells but not of bm12 small B cells (Fig. 4b). In mixtures of bm12 and B6.C20 B cells, no stimulation was observed either in the absence of HETCONJ or T cells, or in the presence of unconjugated $F(ab')_2$ fragments (Fig. 4c). In the presence of HETCONJ and T cells, the number of PFC in small B cells was greatly reduced compared with what was usually seen in total B cells (940 in Fig. 4c versus 9600 in Fig. 3c). IgM^a PFC were more abundant than IgM^b (940 versus 240, linked/bystander ratio = 3·6) (Fig. 4c). Another experiment gave a 2·6 ratio (linked/bystander mean ratio = 3·1 ± 0·71).

Large B6.C20 + bm12 B cells stimulated with LPS showed a somewhat higher representation of IgM^a (Fig. 5a). In the presence of HETCONJ, large B6.C20 B cells showed a high IgM^a response whereas bm12 B cells showed, as predicted, no response (Fig. 5b). Spontaneous IgM production (Fig. 5c) [i.e. in the absence of HETCONJ or in the presence of $F(ab')_2$] was higher (2400 PFC/10^6 B cells) than in unseparated B cells (500 PFC/10^6 B cells, see Fig. 3) or in small B cells (< 100 PFC/10^6 B cells, Fig. 4b), indicating *in vivo* B-cell activation. The B-cell response in the presence of HETCONJ was also higher in large B cells than in unseparated B cells (Fig. 5c, 22880 PFC/10^6 B cells, versus Fig. 3, 9600 PFC/10^6 B cells). The bystander response was greater than in the unseparated B-cell population. (Fig. 5c, 22880 PFC/10^6 B6.C20 B cells, versus 16120 PFC/10^6 bm12 B cells). Linked/bystander ratio was 1·4. Another experiment gave a 1·1 ratio (mean ratio = 1·25 ± 0·21).

DISCUSSION

We have developed a specific heterobifunctional reagent which causes the *in vitro* linking of T cells and B cells in a manner that

may mimic normal T–B collaboration. The reagent consists of the $F(ab')_2$ fragments of two mAb which are covalently bound to each other. One reagent recognizes the epsilon subunit of the CD3 component of the TcR complex. The other mAb is specific for an allelic determinant on the beta chain of a class II MHC molecule. The HETCONJ complex thus can simultaneously bind both to T cells and to genetically appropriate B cells to form a molecular bridge between them.

The current investigation represents the initial characterization of the specificity and efficacy of this HETCONJ reagent. When added to spleen cells of B6 mice, a dose-dependent proliferation was seen which presumably involved mainly T cells. The proliferative effect of the HETCONJ reagent was quantitatively similar to what was seen with the intact IgG anti-CD3 monoclonal antibody. This proliferation apparently required cell–cell interaction. This was demonstrated by the complete failure of $F(ab')_2$ fragments of the anti-CD3 mAb to stimulate proliferation, and by the relative inability of the HETCONJ reagent to stimulate bm12 spleen cells. Presumably, the intact IgG anti-CD3 bound to macrophage and B-cell Fc receptors via its Fc fragment, as well as to the TcR. Similarly, the HETCONJ reagent bound to B cell and macrophage $I\text{-}A^b$ molecules, but not to $I\text{-}A^{bm12}$. It is possible that the weak effect of HETCONJ on bm12 spleen cells was due to a low affinity cross-reaction of the D3-137.5 mAb with the bm12 Ia molecule.

We have also shown that the HETCONJ can mediate *in vitro* T-cell dependent B-cell activation in a specific manner. Purified B6.C20 B cells ($I\text{-}A^b$, Igh^a), in the presence of $(B6.C20 \times bm12)F_1$ T cells, were activated in a dose-dependent manner by the HETCONJ to generate large numbers of IgM^a PFC. As was the case in the proliferation assay, bm12 B cells responded only weakly and inconsistently. This B-cell activation was not induced by a mixture of the $F(ab')_2$ fragments of the anti-CD3 and anti-$I\text{-}A^b$ antibodies and did not occur in the absence of T cells. Although these results demonstrate the specificity of the HETCONJ reagent in B-cell activation, they do not elucidate the mechanism of the effect.

A further set of experiments was, therefore, undertaken to distinguish B-cell activation through the close apposition of T cells and B cells (linked recognition) from that requiring only soluble lymphokines liberated by activated T cells (bystander effect). We mixed B6.C20 and bm12 B cells with F_1 T cells and tested the activating effect of the HETCONJ. In a large number of similar experiments, greater numbers of IgM^a than IgM^b PFC were seen. This differential was not due to sensitivity of the detection system, since LPS-stimulated B6.C20 and bm12 spleen cells, either mixed together or cultured separately, gave comparable numbers of IgM^a and IgM^b PFC, respectively; and $(B6.C20 \times bm12)F_1$ B cells also gave equal numbers of a and b allotype-antibody-forming cells when stimulated with the HETCONJ in the presence of F_1 T cells. These experiments indicate, then, that the B-cell activation mediated by our HETCONJ reagent preferentially involved direct T–B interaction (linked); however, in all experiments a smaller but still significant amount of activation of the non-recognized bm12 B cells was also seen (bystander effect). The approximate relative magnitude of the linked versus the bystander activation was 2:1.

In additional studies, the differential requirements of B-cell subsets for linked versus bystander activation was tested. Large and small B cells were separated by density-gradient centrifugation. The former population presumably had been activated *in vivo*, while the latter cells were considered to be resting. Results indicated that the large B cells were more easily stimulated *in vitro* to produce PFC, as would be expected from their activated status. However, little evidence for T–B linkage preference in their activation mechanism was seen. On the other hand, small B cells gave a 10 times lower response than unseparated B cells. This is in accordance with previous reports[28,29] and reflects the resting status of this population. However, there was a clear preference for linked activation in small B cells, and the strength of this preference was perhaps greater than that of the total B-cell population.

The preference for linked B-cell activation mediated by our HETCONJ reagent is not surprising. Normal T–B interaction would involve recognition of an Ia/antigen complex by the TcR. Our reagent, which links the TcR to Ia, mimics this interaction. Of course, the details of the relative molecular configurations of the TcR and Ia molecule must be somewhat different in the two situations. In addition, our HETCONJ does not include a role of nominal antigen. Antigen may provide an additional signal to the B cell through its interaction with the surface IgM molecules.[26] Furthermore, the Ia/antigen complex may interact with the specific T cell in a manner beyond merely providing a ligand for cross-linking.

According to the model by Noelle and Snow,[30] B cells become lymphokine responsive upon physical contact with T-helper cells. This happens during specific T–B interaction, but also when bystander resting B cells are in the presence of activated T cells.[31] Lymphokines such as IL-2 and IL-5,[32] IL-4[33] or interferon-gamma[34,35] released by T cells probably contribute to B-cell activation. In the case of bm12 B cells, bystander activation cannot involve TcR/Ia cross-linking. It is likely that the anti-CD3 part of the HETCONJ, through the induction of T-cell activation, increases the affinity of LFA-1 for its ligand, ICAM-1.[9] In other systems, T–B interaction through these accessory molecules has been shown to play an important role in *in vitro* activation.[36] Other T-cell surface molecules may also be induced by the activation and explain the non-specific interaction with bm12 B cells and, thus, the bystander effect. In addition, the anti-Ia part of the HETCONJ could inhibit the normal CD4–class II interaction which takes place in a cognate T–B interaction. These various factors may favour bystander activation. However, the consistent preference for linked recognition in our experiments suggests that the HETCONJ not only brought T and B cells into contact, but mimicked some of the events involved in T–B recognition, perhaps through a signal mediated by the $I\text{-}A^b$ molecule. This interpretation is supported by the finding that undigested anti-CD3 induced only one fourth as many PFC as the HETCONJ, and gave an equal number of IgM^a and IgM^b PFC (not shown). In any case, the additional details of the mechanism of cognate interaction are not clear. Specific T–B conjugates show capping of cytoskeletal components of T cells,[37] and local focusing of lymphokine secretion can be induced in response to an anti-TcR antibody.[38] This polarization of the T cell in the process of cognate interaction is likely to be part of an underlying molecular mechanism of T–B collaboration, and may be more efficient when TcR $\alpha\beta$, rather than CD3 subunits, are cross-linked, since anti-TcR $\alpha\beta$ and anti-CD3 mAb have been found to have different effects on T-cell activation.[39]

The HETCONJ can also link T cells to other cells bearing class II MHC molecules, for instance macrophages. It could be

that these effects could lead to increased macrophage activation, etc. However, this is unlikely to be the case with purified resting B cells. While most of the phenomena are understandable as T–B effects, T cells linked to other class II MHC-expressing cells could also be perturbed and affect the results.

The current work extends previous use of heteroconjugate antibodies to link specifically two cell types. In other systems, tumours have been targeted for T-cell cytotoxicity,[40-42] or T-cell help by T-cell clones has been directed at antigen-specific B cells.[13,15] In addition, in one system hybrid antibodies could induce T-cell differentiation in thymic organ culture.[43] The present system permits the direct interaction of polyclonal T cells with polyclonal B cells through a specific T-cell receptor–MHC linking. This allows the use of normal cell populations to explore in more detail further cellular and molecular aspects of T–B collaboration. These possibilities include the role of accessory molecules such as CD4; the cellular polarization mentioned above; the role of immunoglobulin signalling for B-cell activation, as opposed to antigen focusing; the role of lymphokines and the effects of various subsets of B cells and T cells on the efficacy and efficiency of such collaboration.

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