PRIMING OF T HELPER CELLS
BY ANTIGEN-ACTIVATED B CELLS

B Cell-primed Lyt-1+ Helper Cells Are Restricted
to Cooperate with B Cells Expressing the IgvH
Phenotype of the Priming B Cells*

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The humoral immune response to heterologous erythrocytes requires the cooperation of B lymphocytes and helper T cells. The production of anti-sheep erythrocytes (SRBC) IgG is almost wholly dependent on the activity of T help (reviewed in 1). Helper T cells that recognize the carrier portion of an immunogen have been known for some time (2). More recently evidence has accumulated that other helper T cells exist that recognize autologous Ig structures (3) such as allotype (4), isotype (5, 6), or idiotypic (7-10).

Previously it was shown that intravenous transfer of polyclonally stimulated B cell blasts induces activation of syngeneic Lyt-1+,2-,3- T cells (11). Removal of surface Ig (sIg) by capping abolished the capacity of B cell blasts to activate Lyt-1+ T cells. It seemed possible that this activation of Lyt-1+ T cells by Ig-associated structures on B cells might represent the triggering of Ig-specific helper T cells.

The aim of this report is to present findings of experiments testing the hypothesis that antigen-activated B cells display or produce Ig-associated structures that trigger T cells that have receptors specific for these molecules. These triggered T cells would give rise to clones of helper cells specific for B cells displaying Ig-associated molecules similar to those on the priming B cells. To study this, I used B cells from mice recently immunized with SRBC and tried to determine if these B cells could induce T helper cells capable of helping the humoral response to SRBC. I tried to determine whether (a) there is any genetic restriction such that the activating B cells and the helper T cells had to share Ig heavy chain (Igh) alleles or if (b) the specificity of the T helper cells is generated by recognition of structures associated with the priming B cells that are either encoded by genes linked to the loci for the variable (IgVH) or constant (IgCH) part of the immunoglobulin heavy chain.

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1 Abbreviations used in this paper: BSA, bovine serum albumin; C', complement; Cy, cyclophosphamide; FCS, fetal calf serum; HRBC, horse erythrocytes; Igh, immunoglobulin heavy chain; IgCH, genes coding for the constant region of Igh; IgVH, genes coding for the variable region of the Igh; IgCH, variable part of Igh; IgCH, constant part of Igh; PBS, phosphate-buffered saline; PFC, hemolytic plaque-forming cells; RaMIg, rabbit anti-mouse immunoglobulin; RaMIgG, rabbit anti-mouse immunoglobulin G; SRBC, sheep erythrocytes.
Materials and Methods

Mice. 6-8 wk-old female BALB/c (Ig\textsuperscript{a\textsubscript{a}}) mice from Gl.-Bomholtgard, Ry, Denmark on their Ig-congenic partner strains CB17 or CB20 (Ig\textsuperscript{b\textsubscript{b}}) or BAB\textsubscript{14} (Ig\textsuperscript{a-b}) were used. The congenic mice were kindly provided by Dr. M. Simon and Dr. K. Eichmann, Deutsches Krebsforschungszentrum, Heidelberg, Federal Republic of Germany. The genetic background of these strains has been described by Hetzelberger and Eichmann (10).

Antigens. SRBC and horse erythrocytes (HRBC) were obtained from a local source. Whole blood was collected in Alsever's solution weekly from the same animals. Erythrocytes were washed three times in 0.9% NaCl before use.

Antisera. Rabbit anti-mouse IgG (RaMIgG) for developing indirect plaque-forming cells (PFC) was obtained from a single rabbit immunized with purified mouse IgG in complete Freund's adjuvant. Normal rabbit or mouse immunoglobulin was purified by elution from protein A-Sepharose columns (Pharmacia Fine Chemicals, Uppsala, Sweden). Purified rabbit anti-mouse Ig antibodies (RaMIg) were prepared by affinity chromatography of hyperimmune RaMIg sera on a mouse Ig-Sepharose 4B (Pharmacia Fine Chemicals) immunoabsorbent. For coating plastic plates, all Ig preparations were freed from aggregates by centrifugation for 1 h at 100,000 g before use.

Anti-Lyt and rabbit anti-mouse brain (anti-Thy-1) antisera as well as rabbit complement (C') with low background cytotoxicity was kindly provided by Dr. H. Cantor, Harvard Medical School, Boston, Mass., and used as described previously (12, 11).

Preparation of Lymphocyte Populations. Single lymphoid cell suspensions were prepared by teasing excised spleen in phosphate-buffered saline (PBS), pH 7.2, supplemented with 5% heat-inactivated fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.). To remove erythrocytes, cells were incubated for 0.5 min in Tris-HCl-ammonium chloride and washed three times in PBS-FCS.

Enrichment of T Cells. Spleen cells were incubated on RaMIg-coated tissue culture dishes (100 mm; Greiner-C.A., Nürtingen, Federal Republic of Germany) according to Mage et al. (13) and Wysocki and Sato (14) as described earlier (11). Usually, 3 ml of cell suspension in PBS-3% FCS that contained 3 × 10\textsuperscript{7} erythrocyte-free spleen cells were incubated for 30 min at 4°C on precoated plates (30-40 μg RaMIg/plate) decanted, pipetted onto a second plate, and incubated for an additional 30 min. Nonadherent cells were then decanted, washed twice, and counted. The cell recovery was 30-35% of the starting population; Ig-positive cells accounted for <2% of the nonadherent population as assessed by standard immunofluorescence techniques.

Enrichment of B Cells. B cells were prepared by positive selection from RaMIg-coated culture dishes (coated with a mixture of 5-8 μg RaMIg and 50-80 μg normal rabbit Ig) according to the method of Wysocki and Sato (14). The B cells adherent to coated plates in the absence of divalent cations at 4°C were flushed from the plate by pipetting with PBS-FCS.

Enrichment of Antigen-activated B Cell Blasts. Mice were injected with 10\textsuperscript{7} SRBC i.v. 2 d later, spleen cells of 15-20 mice were pooled and depleted of erythrocytes as described above, and 10\textsuperscript{6} of these spleen cells in a volume of 0.1 ml were suspended in 1 ml of 35% bovine serum albumin (BSA; Pathocyte 4; Miles Laboratories, Inc., Elkhart, Ind.) as described earlier (15, 11). This suspension (33% BSA) was placed into a centrifuge tube and overlayed with 1 ml each of 29, 24, and 10% BSA in PBS. After centrifugation at 13,000 g for 30 min at 4°C, which resulted in fractionation of cells according to buoyant density, cells of lowest density (interphase between 10 and 24% BSA) were washed three times in Dulbecco's modified Eagle's medium (DMEM; Flow Laboratories, Inc., U.K) and treated with a 1:40 dilution of rabbit anti-Thy-1 serum plus C' for 1 h. After washing three times, this spleen cell fraction was designated "B cell blast enriched."

Preparation of Macrophage-enriched Populations. Spleen cell suspension of a pool of 10-15 mice immunized 2 d earlier with 10\textsuperscript{7} SRBC were first passed over RaMIg-coated plates in the absence of divalent cations at 4°C as described above. The nonadherent Ig-negative cell fraction, which contained 7-10% macrophages or non-T cells, was resuspended in 10 ml RPMI-1640 medium (Flow Laboratories, Inc.) supplemented with 20% FCS and 3 × 10\textsuperscript{7}-5 × 10\textsuperscript{7} cells were incubated on uncoated culture dishes (100 mm; Greiner-C.A.) for 2 h at 37°C in a humidified CO\textsubscript{2} incubator. The nonadherent cells were discarded and the adherent cells...
harvested by addition of 10 ml ice-cold PBS-5% FCS and vigorously pipetting with a Pasteur pipette.

**Plaque-forming Cell (PFC) Assay.** Direct PFC were assayed using the slide technique of the hemolytic plaque assay (16) as modified by Cunningham and Szenberg (17). Indirect PFC were developed by a 1:2,000 dilution of RaMIgG of a single rabbit. This antiserum did not suppress >5-7% of direct PFC at day 3 of priming with SRBC. The amount of indirect PFC were calculated by subtracting the number of PFC obtained in the absence of developing serum from the number obtained after addition of RaMIgG.

**Experimental Design to Show Induction of T Helper Cells by Antigen-activated B Cells.** Fractions of antigen-activated B cells or B cell blasts were obtained from donors 2 d after immunization with $10^8$ SRBC as described above. $10^7$ cells were injected i.v. into primary recipients that had been pretreated i.p. 1 d before cell transfer with 20 mg/kg cyclophosphamide (Cy) (Endoxan, Asta Werke, Brackwede, Federal Republic of Germany). Their splenic T cells were isolated 2–3 d later and $10^7$ cells injected i.v. into secondary recipients (which were also treated with Cy 1 d previously) together with a priming dose of $10^7$ SRBC. Direct and indirect PFC were determined on day 6.

**Results**

**Transferred B Cell Blasts from SRBC-immunized Mice Induce Augmented Anti-SRBC Responses.** $10^7$ B cell blasts prepared from the spleens of BALB/c mice immunized 2 d earlier with $10^8$ SRBC i.v. were transferred to BALB/c recipients. One-half of these recipients had been given Cy 1 d before. On day 4 or 5, all recipients were given $10^7$ SRBC i.v. and the number of PFC was determined on day 10–11.

As shown in Table I, only Cy-pretreated recipients of SRBC-stimulated B cell blasts showed augmented indirect PFC responses. Injection of B cell blasts into normal recipients resulted in a decrease in indirect PFC. Thus this pilot experiment indicates that (a) there may be helper cell induction by antigen-activated B cell blasts, but (b) this helper activity can be obscured by the presence of Cy-sensitive suppressor cells (18, 19, 11).

**Antigen-activated B Cells Stimulate Helper T Cells.** To demonstrate that the augmented responses found in the preliminary experiments resulted from the expansion of helper T cell clones, a double-transfer experiment was performed. 2 d after BALB/c mice had received $10^8$ SRBC i.v., their spleens were used as a source of B cells, T cells, or

### Table I

Transfer of SRBC-activated B Cell Blasts Induces Augmentation of Anti-SRBC PFC in Syngeneic Recipients Pretreated with Cy

| Donor cells injected i.v. | Treatment of recipient | n§ | Anti-SRBC PFC per recipient spleen || Direct | Indirect |
|--------------------------|-----------------------|----|----------------------------------|--------|---------|
| None                     | None                  | 5  | $76.3 \pm 7.6$                   | $42.7 \pm 4.4$ |
| None                     | Cy                    | 6  | $62.1 \pm 5.8$                   | $38.8 \pm 5.2$ |
| SRBC-activated B cell blasts | None              | 5  | $73.2 \pm 5.9$                   | $5.1 \pm 0.9$  |
| SRBC-activated B cell blasts | Cy              | 7  | $105.7 \pm 12.7$                 | $180.3 \pm 14.5$ |

* $10^7$ B cell blasts separated from a pool of 20 BALB/c spleen donors 2 d after administration of $10^8$ SRBC i.v. The blasts were transferred i.v. 4–5 d before immunization of recipients.

‡ 20 mg/kg Cy was given i.p. 1 d before cell transfer.

§ Number of recipients in each group.

\| Data represent the mean ± SE of the numbers of splenic PFC in groups of BALB/c recipients 6 d after immunization with $10^7$ SRBC i.v. (data of two independent experiments pooled).
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CELLS TRANSFERRED into:

| GROUP | (1.recipient) | (2.recipient) |
|-------|---------------|---------------|
| A     | control       | control       |
| B     | none          | TR1           |
| C     | 10⁶SRBC       | TR1           |
| D     | 10⁶SRBC       | TR1           |
| E     | 1°T cells     | TR1           |
| F     | 1°Macrophages | TR1           |
| G     | 1°B cells     | TR1           |
| H     | 1°B blasts    | TR1           |
| J     | 1°B blasts    | (anti Lyt-1 + C') |

FIG. 1. Demonstration of helper T cell induction by antigen-activated B cells in syngeneic double-transfer experiments. Fractions of antigen-activated spleen cells (as described in Material and Methods) were obtained from BALB/c donors 2 d after 10⁶ SRBC i.v. (primed [1°] donors). 10⁷ cells were injected i.v. into first BALB/c recipients (R1). Their splenic T cells were isolated 2-3 d later and 10⁷ cells injected i.v. into secondary recipients (R2) together with a priming dose of 10⁷ SRBC. All R1, R2, and controls had been pretreated with 20 mg/kg Cy 1 d before cell transfer. Direct and indirect PFC were determined in groups of R2 6 d after priming. Mean and SE of PFC in R2 are presented as the percentage of that found in control group A (three to five R2 in each group). Cells transferred to R1: group A and B, none; C, no donor cells + 10⁶ SRBC; D, same as C, but + 10⁶ SRBC; E, T cells of 1° donors; F, macrophages of 1° donors; G, B cells of 1° donors; H, B cell blast-enriched fraction of 1° donors; J, same as H, but T cells of R1 treated with anti-Lyt-1 + C'.

macrophage-enriched populations. These were transferred into primary BALB/c recipients that had been given Cy 1 d earlier. On day 2 or 3, splenic T cells from the primary recipients were transferred together with 10⁷ SRBC i.v. into secondary BALB/c recipients that had also been Cy pretreated. On the 6 d after transfer, direct and indirect PFC were determined.

As shown in Fig. 1, T cells from mice that had been given SRBC-activated B cells (group G) or B blasts (group H) produced significantly more indirect PFC than T cells of primary recipients of no cells (group B), activated T cells (group E), activated macrophages (group F), or two different doses of SRBC (groups C and D). Anti-Lyt-1 + C'-treated T cells of primary recipients of antigen-activated B blasts failed to cooperate in their secondary hosts (group I); the same was true for anti-Thy-1 + C'-treated T cells (data not shown).

The double-transfer experiments indicate that within 2 d after activation by antigen, B cells or B cell blasts express structures that stimulate syngeneic T cells with Lyt-1+ phenotype—which in their effector phase can help syngeneic B cells to produce more anti-SRBC antibodies of IgG classes (indirect PFC).

Induction of T Cell Help by Activated B Cells is Antigen Specific. In experiments analogous to those described above (Table I), SRBC-immunized BALB/c mice were the source of splenic B cell blasts, which were enriched on density gradients and treated with anti-Thy-1 and C'. These B cell blasts were transferred to syngeneic recipients that had been pretreated with 20 mg/kg Cy 1 d earlier. 4-5 d later, the
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Table II

Induction of Help in Recipients of SRBC-activated B Cell Blasts is Antigen Specific

| Igh phenotype of Donors* | PFC per recipient spleen§ | Anti-SRBC | Anti-HRBC |
|--------------------------|---------------------------|-----------|-----------|
| Ighv Ighc Ighv Ighc | Direct | Indirect | Direct | Indirect |
| a a | 52.6 ± 9.6 | 22.1 ± 4.8 | 54.6 ± 3.7 | 26.9 ± 3.6 |
| a a | 125.4 ± 10.6 | 218.3 ± 15.2 | 51.2 ± 3.5 | 20.1 ± 2.9 |

* B cell blasts were separated from 14-20 pooled BALB/c spleens 2 d after immunization of donors with 10⁶ SRBC as described in Materials and Methods.

§ Data represent the mean ± SE of the numbers of PFC determined in the spleens of 4-6 recipients per group 6 d after immunization with either 10⁷ SRBC or HRBC.

Table III

Augmentation of Indirect PFC Can Only Be Demonstrated in Recipients of SRBC-activated B Cell Blasts That Share the Ighv Phenotype with Donors

| Igh phenotype of Group | Anti-SRBC PFC per recipient spleen§ | Donor* | Recipient‡ | Direct | Indirect |
|------------------------|------------------------------------|-------|------------|-------|---------|
| Ighv Ighc Ighv Ighc | × 10⁻³ | a a | a a | 49.3 ± 5.4 | 29.4 ± 9 |
| B a | 78.8 ± 6.2 | 38.3 ± 5.2 |
| C b | 71.4 ± 6.1 | 38.3 ± 3.9 |
| D a b | 112.0 ± 7.8 | 187.2 ± 22.6 |
| E a a b | 101.7 ± 8.1 | 195.9 ± 20.2 |
| F a b b | 68.5 ± 9.6 | 45.5 ± 7.1 |

* Splenic B cell blasts from BALB/c donors (a/a) were isolated 2 d after immunization of donors with 10⁶ SRBC (15-20 spleens pooled/experiment).

‡ 10⁷ blasts were injected i.v. into either BALB/c, BAB14 (a/b), CB17 or CB20 (b/b) recipients (given Cy i.p. 1 d previously) 4-5 d before immunization of recipients (3-5 recipients per group).

§ Data represent the means ± SE of the numbers of splenic PFC determined 6 d after immunization of recipients with 10⁷ SRBC i.v.

|| Data of three CB17 and four CB20 recipients pooled.

As shown in Table II, only mice that received SRBC-activated B cell blasts and SRBC showed augmented indirect PFC. Those that received SRBC-activated B blasts and HRBC had responses no better than controls. This experiment rules out the possibility that SRBC-activated B cells activate an unspecific T helper population. However, this experiment does not rule out the more complex possibility that optimal T cell help requires that both SRBC and HRBC be given to the recipients.

Transferred B Cells Differing from Recipients in Their Ig Heavy Chain Variable Regions Do Not Induce Augmented Responses. The protocol used in these experiments was the single transfer system described in Tables I and II. Here, the source of B cell blasts was always spleen cells of BALB/c donors immunized to SRBC, however, the recipients...
 were varied so that some were Ig-syngeneic BALB/c (Ighv: a, Ighc: a,) others were, congeneric for the heavy chain haplotype (CB 17 or CB20: Ighv: b, Ighc: b; BAB 14: a, Ighc: b). As shown in Table III, when the B cell blasts and the recipients were syngeneic (group D) or Ighv sharing (BAB14) (group E) a four- to ninefold augmentation in the number of indirect PFC resulted. B cell blasts from Igh congenic mice (CB 17 or CB 20) failed to augment (group F). These results suggest that the increase of specific indirect PFC is restricted to the situation in which the Ighv phenotype of activating cells and the recipient are the same.

The Igh Phenotype of Activated B Cells That Induce T Helper Cells Must Match That of the Responding B Cells. In the previous experiments (Table III) it was not possible to decide whether the matching of B cell blasts and recipients took place at the level of B induction of helper T cells or whether T cells can be primed to cooperate with B cells carrying the signalling Igh phenotype. To test this, I did criss-cross double transfers of antigen-activated B cells of different Ighv phenotypes with Igh syngeneic or congenic first and second recipients. The basic design of the experiments was, similar to that presented in Fig. 1 for group G. As shown in Fig. 2 syngeneic (group F) or Ighv syngeneic (group G) B cells augment, indirect PFC production. Furthermore B cells will prime Igh-congenic T cells if the triggering B cells and the antibody-producing B cells share Ighv phenotypes (group H). Igh identity of triggering B cells and helper T cells is not sufficient to generate help when there is a mismatch with the B cells of the antibody producer (group I and L).

In analogy to the restriction for Ighv, syngeneity seen in the single-transfer
experiments (Table III, group F) T cells stimulated by Igh-congeneric B cells do not help B cells of their own IgvH genotype (group K).

These experiments suggest no primary genetic restriction for T-B collaboration of Igh-congeneric strains, but rather indicate that a set of T cells can recognize IgvH-encoded structures of activated B cells. In their effector phase, those primed T helper cells cooperate in a restricted fashion only with B cells expressing the priming Ighv structures.

Discussion

Since the importance of the role of regulatory T cells in the humoral immune response was first understood, B cells have been viewed as acceptors of T cell-generated signals. These T cell signals are seen as regulators of the quality and quantity of antibody produced by the foot-soldier B cells.

The studies reported here show that within 2 d after the introduction of antigen, SRBC-activated B cells can deliver a signal to T cells that prime these T cells to become helper cells. In their effector phase, these T cells cause augmented production of IgG antibodies by B cells carrying structures similar to those on the priming B cells.

The results presented in Table III and Fig. 2 suggest that the relevant signals for this B-T-B communication are encoded for by genes linked to—or part of—the IgvH region. In previous studies (11), it was shown that B cell activation of syngeneic T cells requires cell surface Ig- or Ig-associated molecules. The results of this report suggest strongly that IgvH-locus-encoded structures, rather than non-Ig products of closely linked genes, supply the relevant signals. This implies that the help generated must be antigen specific. This prediction could be confirmed for the non-cross-reacting antigens SRBC and HRBC. However the more complex possibility that unspecific or isotype-specific helpers were primed but failed to help in the primary response to HRBC because they still required restimulation by the priming antigen to express their help has not been ruled out.

The restriction for IgvH syngenicity demonstrated here seems to contrast with earlier studies (20) that had revealed no Igh-linked restriction of T cell help for B cells in the IgG2a response to SRBC. In these studies, the Igh-congeneric pair of CSW and CWB mice was used in a design in which thymus cells were transferred into thymectomized, irradiated, bone marrow-reconstituted, Igh-congenic recipients. Unlike the current experiments, the priming as well as the challenge dose of antigen was given after the T and B cells had been placed together. Thus any restriction that took place would be to the mismatched B cell Igh phenotype.

This view is supported by results of T cell reconstitution experiments done in athymic (nude) CBA mice with T cells from a pair of Igh-congeneric CBA mice as reported by Wortis et al. (21) and Nutt et al. (22). Igh-matched as well as unmatched T cells provide equal help for production of all isotypes of anti-SRBC antibodies if priming with SRBC was performed in the nude recipients after T cell transfer. However transfer of T cells already primed in the environment of either Igh-matched or -unmatched B cells could provide help only for those B cells that express the Igh phenotype of the priming environment.

The hypothesis that emerges is that B cells that express Ig-containing structures prime a set of T cells that can help B cells that bear Ighv-matching structures. Under these conditions, there is no genetic restriction of T-B or B-T collaboration, only the
need for B-B matching. Whether there are additional major histocompatibility complex-imposed restrictions is currently under study.

The relationship of classical, carrier-specific T help and Ig-specific T help needs further elaboration. As a working hypothesis, it is suggested that: carrier-specific helper T cells drive the proliferation and/or differentiation of B cells to a stage where they express the Ig structures that trigger the Ig-specific T helper cells (TH\textsubscript{H\textsubscript{G}}). The thus-primed TH\textsubscript{H\textsubscript{G}} then augment and/or determine the quality of the B cell response, resulting in an increased production of antibodies of IgG classes.

In this paper the mechanism by which the Ig\textsubscript{H\textsubscript{G}}-locus-encoded structures of antigen-activated B cells lead to priming of TH\textsubscript{H\textsubscript{G}} has not been established. Several questions need further study: (a) Do soluble products stimulate (either via secretion or by shedding from activated B cell surface)? (b) Is direct B-T cell contact necessary? (c) Do TH\textsubscript{H\textsubscript{G}} have to see antigen in association with the priming Ig\textsubscript{H\textsubscript{G}} structures? Findings reported by Forni et al. (23) suggest an antigen-independent but T cell-dependent mechanism of induction of antibody response by specific Ig as a network regulation (24) among elements of the immune system itself. In their experiments, Forni et al. have shown that injection of monoclonal IgM-antibodies directed against thymus-dependent and thymus-independent antigens can induce antibody production of the respective specificity in normal but not in athymic (nude) mice.

In conclusion, I would like to suggest the following hypothesis on the nature and sequential events of B-T-B cell interactions after perturbation of the immune system by heterogeneous antigens (e.g., SRBC): After recognition of antigen (macrophage processed?) by B cells via their specific cell surface receptors, B cells react by forming blasts by proliferating and/or differentiating. The activated B cells display an Ig\textsubscript{H\textsubscript{G}}-linked gene product that is recognized by a complementary receptor (e.g., anti-idiotope) on a set of helper T cells. This recognition leads to clonal expansion by differentiation and/or proliferation to TH\textsubscript{H\textsubscript{G}} effector cells. These TH\textsubscript{H\textsubscript{G}} are thus primed or adaptively restricted to help antigen-activated B cells with an Ig\textsubscript{H\textsubscript{G}} phenotype similar to the priming B cells. Net effect of this TH\textsubscript{H\textsubscript{G}}-mediated help is augmented production of specific antibodies of IgG classes.

Future studies will provide more information on the repertoire and nature of the TH\textsubscript{H\textsubscript{G}}-receptor, the induction and duration of TH\textsubscript{H\textsubscript{G}} memory, the role of carrier-specific help, and the role of suppressive regulatory mechanisms involved in the described phenomenon.

Summary

Activated B cells isolated shortly after primary immunization of BALB/c donor mice with sheep erythrocytes (SRBC), were transferred to normal syngeneic recipients or to low-dose cyclophosphamide-pretreated syngeneic recipients. In pretreated recipients, the transfer of activated B cells, but not of T cells or macrophages, resulted in an augmented production of indirect plaque-forming cells in the primary immune response to SRBC but not to horse erythrocytes. It was shown in double-transfer experiments that T helper cells (Lyt-1\textsuperscript{+}) had been stimulated by the transfer of antigen-activated B cells. Criss-cross double-transfer experiments using the mouse strains CB20 and BAB14 (congenic to BALB/c at the loci coding for the immunoglobulin heavy chain) indicate that those T helper cells are primed after recognition of B cell products that are encoded for by genes linked to the loci coding for the
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variable region of the immunoglobulin heavy chain (IgvH). The thus-primed Ig-dependent T helper cells (TH9g) are adaptively restricted to cooperate with B cells that display IgvH-linked gene products similar to those that originally stimulated the TH9g. These findings suggest that in the course of an immune response to T cell-dependent antigens, help for the production of specific IgG can be provided by TH9g that have been primed and/or clonally expanded after recognition of IgvH-linked gene products by (e.g., complementary) T cell receptors.

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