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Studies on B-cell memory

III. T-DEPENDENT ASPECT OF B MEMORY GENERATION IN MICE IMMUNIZED WITH T-INDEPENDENT TYPE-2 (TI-2) ANTIGEN

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Summary. The time course of B-cell memory development to a dinitrophenyl (DNP) T-independent type-2 (TI-2) antigen was investigated by adoptive cell transfer. Strong IgM and IgG memory developed in BALB/c mice after immunization with DNP-dextran, to be recalled by challenge with either T-dependent (TD) antigen or TI-2 antigen. However, only weak IgM memory and very feeble IgG memory were detected in athymic nude mice receiving the same immunization as euthymic mice. Once memory was established under probable T cell influence, its recall by TI-2 antigen challenge seemed independent of T cell help and did not require sharing of carriers between priming and challenge antigens.

The following may be concluded. (i) Long-term IgM and IgG memory is induced by TI-2 antigen priming in the presence of functional T cells. (ii) The class switch from IgM to IgG in the memory B cell pool is driven effectively by TI-2 antigen and is probably T cell-dependent.

INTRODUCTION

Since the pioneering work of Claman, Chaperon & Triplett (1966) and of Michell & Miller (1968), it has been well established that a cooperative interaction between thymus-derived T cells and antibody-producing B cells is required for optimal antibody responses to most antigens. However, such a T–B co-operation is not necessarily required for the induction of B cell memory (Roelants & Askonas, 1972; Hosokawa, Amagai & Muramatsu, 1979). Studies with allotype-suppressed mice established that IgG memory B cells are elicited by priming with TD antigen in the absence of mature T cells, but that the differentiation of IgG memory cells into antibody-forming cells (AFC) is dependent on T cell help if TD antigens are used for challenge (Okumura *et al.*, 1976).

On the other hand, it has been believed that T-independent (TI) antigens and hapten-conjugated TI antigens do not induce long-term memory. However, some investigators have reported substantial IgM memory to TI antigens. Thus, TI-2 antigens, which are distinguishable from T-independent type-1 (TI-1) antigens by their inability to elicit antibody responses in CBA/N mice, are able to induce IgM memory B cells which are activated to generate IgM AFC in response to a challenge with TD, TI-1 or TI-2 antigens (Guercio, Thobie & Poirier, 1974; Diaz-Espada, Martinz-Alonso & Bernabe, 1978; Hosokawa, 1979). The manifestation of such IgM memory by challenge with TI-2 antigens is independent of T cell help, since it is driven even in the absence of mature T cells (Hosokawa, 1979).

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Whether or not TI-2 antigens induce IgG memory B cells is still unclear. Many investigators including us failed to detect IgG memory cells in animals primed with TI-2 antigens (Baker *et al.*, 1979; Guercio *et al.*, 1974; Hosokawa, 1979), although a few investigators detected only weak IgG memory to TI-2 antigens (Schott & Merchant, 1979; Umetsu, Chapman-Alexander & Thorbecke, 1979). TI-2 antigens were reported to elicit substantial amounts of IgG antibodies in unprimed mice (Sharon *et al.*, 1975). Therefore, 'secondary' IgG responses of primed cells should be carefully compared with primary IgG response of unprimed control cells. Recently, Colle, Motta & Truffa-Bachi (1983) have reported that substantial IgG memory to a typical TI-2 antigen DNP-Ficoll was detected 7 days after priming by challenge with a TI-1 antigen. The reason why some investigators failed to detect IgG memory to TI-2 antigens and others detected it may be ascribed to the time course of IgG memory generation. It is possible that the IgG memory observed by Colle *et al.* (1983) was not a long-term memory but a transient one. Therefore, intensive studies on the immunization method, on the time interval between priming and challenge and on the assay system for memory cells should be performed in order to draw any conclusions on IgG memory to TI-2 antigens.

In this report, the main aim has been to investigate the induction and expression of IgG memory by TI-2 antigens in athymic and euthymic mice. The data presented below show that strong and long-term IgM and IgG memory is induced by priming with the TI-2 antigen DNP-dextran in the presence of T cells, but that the expression of such memory is independent of T-cell help.

MATERIALS AND METHODS

Mice

Inbred BALB/cCr (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka), BALB/c-nu/+, BALB/c-nu/nu (Clea Japan Inc., Tokyo) mice, 2–3 months old were used.

Antigens

Keyhole limpet haemocyanin (KLH) (Calbiochem., San Diego, CA) was conjugated with 2,4-dinitrophenyl (DNP) by the method of Eisen *et al.* (1959). Dextran T-2000 (DE) (Parmacia, Uppsala) and Ficoll 400 (Ficoll) (Pharmacia, Uppsala) were dinitrophenylated as described previously (Hosokawa, 1979). Thus, we prepared DNP_{24}-KLH, DNP_{22}-DE and DNP_{30}-Ficoll (each subscript represents the number of DNP molecules per 4×10^5 mol. wt. of each carrier molecule).

Assay for B cell memory

Mice were immunized by an i.p injection of DNP-DE (20 μg) or DE (20 μg) in 0·2 ml of Freund's incomplete adjuvant (FIA) emulsion. One to 15 weeks after the priming immunization, their spleens were removed to prepare cell suspensions in Eagle's MEM. In some experiments, spleen cells were depleted of T cells by treatment with monoclonal anti-Thy-1·2 antibody (Olac, Bicester, Oxon, U.K.) plus guinea-pig complement. Aliquots of spleen cell suspension thus prepared containing $2–2{\cdot}4 \times 10^7$ cells were transferred i.v. into irradiated (600 rads) syngeneic mice.

Carrier-primed mice immunized with 100 μg of KLH in Freund's complete adjuvant (FCA) 2 weeks before the cell transfer were used as the recipients for the TD challenge with 30 μg of DNP-KLH in saline, and normal mice were used as the recipients for the TI-2 challenge with 10 μg of DNP-DE or 10 μg of DNP-Ficoll in saline. Recipients were challenged about 1 hr after the cell transfer, and their spleens were assayed for plaque-forming cells 6 days later, unless otherwise indicated.

Haemolytic plaque-forming cell (PFC) assay

Cells secreting antibodies to DNP were enumerated by the direct and the indirect methods of plaque assay in agarose gel on microscope slides as previously described (Hosokawa, 1979). Indirect PFC numbers were obtained by subtracting the number of PFC developed with complement alone from the number of PFC developed with a facilitating antiserum and complement. Sheep erythrocytes (SRBC) trinitrophenylated by the method of Rittenberg & Pratt (1969) were used as target cells in the PFC assay.

The facilitating antisera for PFC assay were prepared as previously described (Hosono & Muramatsu, 1972). The antiserum used in this study was effective in facilitating the formation of haemolytic plaques by MOPC-J606 (IgG3, kindly donated from Dr S. Migita, Kanazawa University), MOPC-21 (IgG1) and X5563 (IgG2a) myeloma cells in agarose gel containing Protein A-coated SRBC as target cells. Optimal dilution of the antiserum ($\times 300$) was determined to obtain the maximum number of indirect PFC and thus diluted antiserum did not inhibit the formation of direct PFC.

Statistical analysis

Student's *t*-test was used to determine the significance of difference between experimental groups. Differences were considered significant if *P*-value was less than 0·05.

RESULTS

Kinetics of adoptive secondary responses of DNP-DE-primed spleen cells elicited by DNP-DE or DNP-KLH challenge

Spleen cells ($2{\cdot}4 \times 10^7$) from BALB/c mice primed to DNP-DE or DE 2 weeks previously were transferred i.v. into recipients for TD challenge and recipients for TI-2 challenge. About 1 hr after cell transfer, the KLH-primed recipients were challenged with DNP-KLH and the normal recipients with DNP-DE, respectively. PFC in the recipient spleens were assayed 3–13 days after cell transfer. Recipients of DE-primed spleen cells served as controls. Results are shown in Fig. 1. Mice receiving DNP-DE-primed cells gave remarkable anamnestic IgM responses and significantly higher IgG responses to either TD or TI-2 challenge as compared with mice receiving DE-primed cells (Fig. 1). The peak PFC responses of both IgM and IgG appeared 5–6 days after challenge. On the other hand, mice receiving DE-primed spleen cells showed no clear peak responses. Therefore, PFC assays in the further experiments were carried out 6 days after cell transfer.

Time course of B cell memory generation in mice immunized with DNP-DE

Eight-week-old BALB/c mice were divided into two donor groups. Each group of mice was immunized i.p. with either DNP-DE or DE in FIA. At various time intervals after priming, spleen cells of five mice in each group were pooled. Aliquots of pooled spleen cells ($2{\cdot}4 \times 10^7$) were transferred i.v. into recipients for TD or TI-2 challenge. Recipients were challenged as described above. Results are shown in Fig. 2.

Strong IgM and IgG memory was detected by challenge with either TD or TI-2 antigen. IgM memory appeared as early as 1 week, continued to

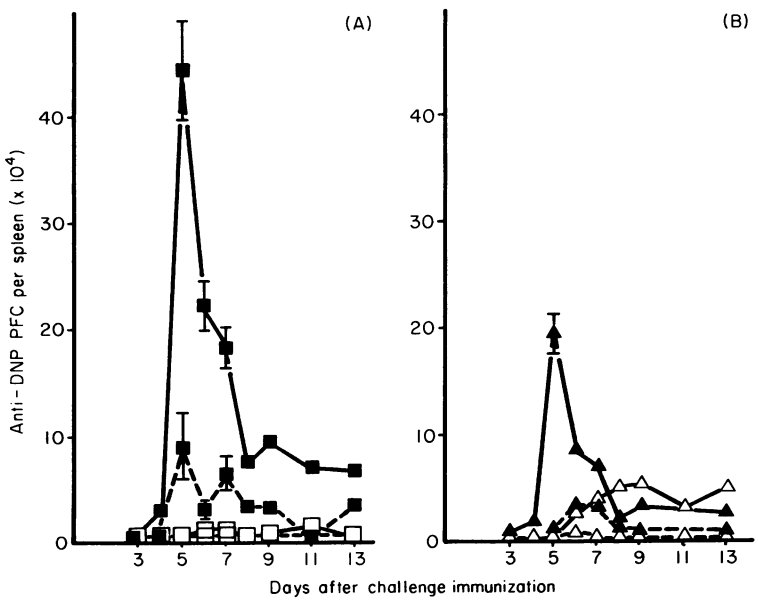

Figure 1. Adoptive antibody responses of DNP-DE-primed (*closed symbols*) and DE-primed (*open symbols*) spleen cells elicited by either challenge of DNP-KLH (A) or DNP-DE (B). Aliquots of spleen cells ($2{\cdot}4 \times 10^7$) from BALB/c mice primed to DNP-DE or DE 2 weeks previously were transferred into recipients for DNP-KLH challenge and were challenged about 1 hr later. Direct (*solid line*) and indirect (*broken line*) PFC numbers in recipient spleens were measured 3–13 days after challenge. Each point and bar represents mean ± SE of three or four recipients.

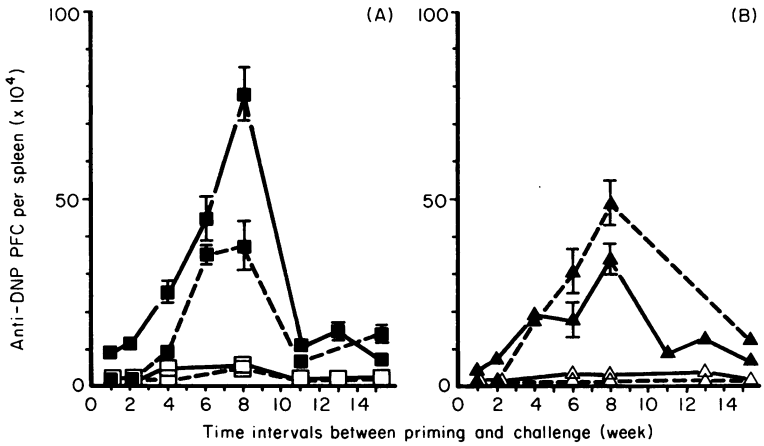

Figure 2. Time course of B cell memory generation to DNP-DE in BALB/c mice. Donors primed with DNP-DE (*closed symbols*) or with DE (*open symbols*) were killed to remove spleens various time intervals after priming (*abscissa*). Aliquots of spleen cells ($2{\cdot}4 \times 10^7$) were transferred into recipients for DNP-KLH challenge (A) and for DNP-DE challenge (B) and were challenged about 1 hr later. Direct (*solid line*) and indirect (*broken line*) PFC numbers in recipient spleens were measured 6 days after challenge. Each point and bar represents mean ±SE or five to eight recipients.

expand until 8 weeks after priming, and declined gradually thereafter. The IgM memory lasted for at least 15·5 weeks (the longest time interval examined). The IgM memory seemed to be recalled by TD challenge much better than by TI-2 challenge.

Clear IgG memory was first detected 4 weeks after priming by either TD or TI-2 challenge and followed time courses similar to those of IgM memory. At the peak of IgG memory level, IgG PFC numbers of experimental groups were 378,300±64,200 and 484,100±60,000 per recipient spleen (6·8 times and 372·4 times higher than those of control groups) for TD challenge and for TI-2 challenge, respectively.

B cell memory generation under athymic conditions

Since DNP-DE mixed with FIA was found to be a potent immunogen to induce IgG as well as IgM memory in euthymic BALB/c mice (Fig. 2), we attempted to induce IgG memory in nude mice by immunization with DNP-DE in IFA. Spleens were removed 7 or 10 weeks after immunization, since maximum memory was generated in euthymic BALB/c mice 8 weeks after priming. Aliquots of spleen cells ($2{\cdot}4 \times 10^7$) were transferred i.v. into BALB/c−nu/+ recipients for TD challenge and for TI-2 challenge as described above.

Results shown in Table 1 indicate that DNP-DE induced IgM memory in nude mice. The memory was recalled by TD challenge as well as TI-2 challenge. The magnitude of the memory, however, was much smaller than that in euthymic mice. On the other hand, DNP-DE mixed with FIA seemed to induce only feeble, if any, IgG memory in nude mice. The manifestation of the meager IgG memory appeared dependent on T cell help, since only TD challenge, but not TI-2 challenge, elicited significantly augmented IgG PFC response in recipients which had received spleen cells primed 7 weeks previously (Table 1, Exp. 1).

T cell-independent activation of DNP-DE-primed B cells by TI-2 antigen

The manifestation of IgG memory generated by TD priming in the absence of mature T cells is dependent on T cell help (Okumura *et al.*, 1976). Therefore, we checked whether memory B cells generated in DNP-DE-primed euthymic BALB/c mice require T cell help for their activation by DNP-DE. Spleen cells primed to DNP-DE were treated with monoclonal anti-Thy-1·2 antibody plus complement, treated with complement alone, or left untreated. Aliquots of cell suspensions ($2{\cdot}4 \times 10^7$) were transferred i.v. into irradiated recipients for TI-2 challenge. Challenge and

Table 1. B cell memory generation by DNP-DE priming in athymic nude mice

Cells transferred†	Recipients challenged with‡	Anti-DNP PFC per spleen§			
		Exp. 1		Exp. 2	
		Direct	Indirect	Direct	Indirect
DNP-DE-primed	DNP-KLH	53,800±6150***	21,700±6000*	92,900±16,100***	11,000±6870
DE-primed	DNP-KLH	18,100±3590	3260±1790	23,600±3280	3000±1620
DNP-DE-primed	DNP-DE	60,400±7710**	2800±1630	59,400±2430**	80±70
DE-primed	DNP-DE	23,800±4310	467±426	36,900±3900	3270±1220

† Nude mice were immunized with 20 μg DNP-DE in FIA or 20 μg DE in FIA. Seven weeks (Exp. 1) or 10 weeks (Exp. 2) after the immunization, spleens were removed and $2{\cdot}4 \times 10^7$ spleen cells were transferred into 600 rads irradiated nu/+ recipients.

‡ Nu/+ mice primed with 100 μg KLH in FCA 2 weeks previously were used as recipients for TD challenge with 30 μg DNP-KLH in saline and unprimed nu/+ mice were used as recipients for TI challenge with 10 μg DNP-DE in saline. PFC assays were carried out 6 days after cell transfer.

§ Arithmetic mean ±1 SE. Statistically significant differences between DNP-DE-primed group and DE-primed group in the same challenge groups: $*P<0{\cdot}05$; $**P<0{\cdot}01$, $***P<0{\cdot}001$.

PFC assays were carried out as above. Results are shown in Fig. 3. Both IgM and IgG B memory cells from DNP-DE primed BALB/c mice were activated by challenge with DNP-DE irrespective of the treatment of the donor cells.

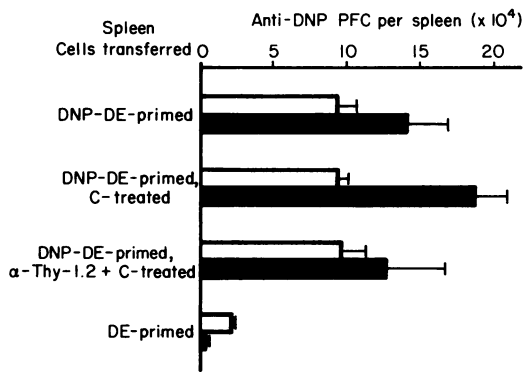

Figure 3. Activation of DNP-DE-primed B memory cells by DNP-DE in the absence of T cells. Spleen cells primed to DNP-DE 10 weeks previously were left untreated, treated with complement alone, or treated with monoclonal anti-Thy-1·2 antibody+complement. Aliquots of cells ($2{\cdot}4 \times 10^7$) thus treated were transferred into recipients for DNP-DE challenge and were challenged about 1 hr later. Spleen cells primed to DE were served as control. Direct (*open column*) and indirect (*solid column*) PFC numbers in recipient spleens were measured 6 days after challenge. Each column and bar represents mean ±SE of four to seven recipients.

Activation of DNP-DE-primed B cells by immunization with DNP-coupled heterologous carrier polysaccharide

The results shown in Fig. 3 strongly suggest that the activation of DNP-DE-primed memory B cells by TI-2 antigen is independent on T cell help. However, a possibility that T-independent and carrier-specific helpers may take part in B cell memory activation (Schott & Merchant, 1979) still remains. In order to check this, DNP-DE-primed spleen cells were transferred i.v. into recipients for TI-2 challenge and the recipients were challenged with DNP-Ficoll. PFC assays were carried out as above. Results are shown in Table 2. The challenge with DNP-Ficoll was as effective as that with DNP-DE in recalling both IgM and IgG memory generated by DNP-DE priming.

DISCUSSION

The present study has demonstrated the following points. A typical TI-2 antigen DNP-DE induces very strong IgM and IgG memory in euthymic BALB/c mice. However, in athymic nude mice only feeble IgG memory is induced by TI-2 priming, although substantial magnitude of IgM memory is induced. Once memory is established in the presence of T cells, the activation of both IgM and IgG memory cells by TI-2 challenge is barely dependent on T cell help and of possible carrier-specific non-T cell help.

In our previous work (Hosokawa, 1979), IgG

Table 2. Carrier independent activation of memory B cells generated by DNP-DE immunization

Cells transferred†	Recipients challenged with‡	Anti-DNP PFC per spleen§			
		Exp. 1		Exp. 2	
		Direct	Indirect	Direct	Indirect
DNP-DE-primed	DNP-DE	92,200 ± 9950***	52,900 ± 6200***	92,900 ± 15,900**	143,700 ± 26,000***
DNP-DE-primed	DNP-Ficoll	83,400 ± 4130**	36,600 ± 10,200**	91,700 ± 14,300***	173,200 ± 31,400***
DE-primed	DNP-DE	33,100 ± 4650	2290 ± 1380	21,800 ± 5060	2600 ± 2200
DE-primed	DNP-Ficoll	25,900 ± 3690	3310 ± 1610	16,900 ± 3950	8300 ± 6040

† BALB/c mice were injected with 20 μg DNP-DE in FIA or 20 μg DE in FIA. Ten weeks after the injection, spleens were removed and $2{\cdot}4 \times 10^7$ spleen cells were transferred into 600 rads irradiated BALB/c mice.

‡ Unprimed irradiated BALB/c mice were used as recipients for either DNP-DE (10 μg in saline) challenge or DNP-Ficoll (10 μg in saline) challenge. PFC assays were carried out 6 days after cell transfer.

§ Arithmetic mean ± 1 SE. Statistically significant differences between DNP-DE-primed group and DE-primed group in the same challenge groups: $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

memory was not detected in BALB/c mice which had received four priming injections of DNP-DE in saline every 4 days. Cell transfer and challenge were carried out 26 days after the first priming injection. Therefore, we missed the rising phase as well as the peak point of IgG memory development, since in the present work clear IgG memory was first detected 4 weeks after the priming and it reached the peak 8 weeks after the priming.

In the present work, the adjuvant may take an important part in inducing IgG memory cells to TI-2 antigens. Usually, TI-2 antigens dissolved in physiological saline or in phosphate-buffered saline are injected into animals without adjuvant. Immunization with TI-2 antigens in soluble form may be an unsuitable method to induce IgG memory cells.

Colle *et al.* (1983) have demostrated that substantial IgG memory was generated by TI-2 priming in the absence of functional T cells. Thus, unprimed spleen cells depleted of Thy-1-positive cells were primed with a TI-2 antigen DNP-Ficoll in irradiated syngeneic recipients and IgG memory was detected by challenge with a TI-1 antigen DNP-LPS 7 days after the priming. Their results do not appear consistent with our present results. However, it is possible that the IgG memory observed by Colle *et al.* was transient memory and different from the long-term memory in our present work. We also observed that such early memory (a priming-like phenomenon) was induced in the absence of functional T cells (T. Hosokawa, unpublished observation). Thus, spleen cells from normal nude mice, which had received a first immunization with DNP-DE in irradiated nu/+ recipients, showed enhanced IgM and IgG PFC responses when challenged with DNP-KLH in the presence of KLH-specific helper T cells 1 or 2 days later.

A carrier-specific immune memory to a TI-2 antigen was reported by Schott & Merchant (1979). Thus, both athymic (nu/nu) mice and euthymic (nu/+) mice, which had been primed with DNP-Ficoll or Ficoll 7 days before challenge, showed enhanced anti-DNP PFC responses of IgM and IgG classes only when challenged with DNP-Ficoll. Our present results appear inconsistent with this, since the activation of IgM and IgG memory cells in our experiments is independent of the sharing of carriers between priming and challenge antigen. The apparent inconsistency may be ascribed to the difference in the time intervals between priming and challenge. The carrier-specific memory was detected 7 days after priming by DNP-Ficoll challenge *in situ*. There was no information about the time course of the memory development. Therefore, it is possible that only a transient phenomenon was detected as carrier-specific memory or that such memory hid itself behind the large magnitude of secondary responses of carrier-independent memory to the challenge in our experiments.

The expression of the IgM and IgG memory, which was generated by priming with DNP-DE in BALB/c mice, seems independent of T cell help (Fig. 3).

However, a possibility that radio-resistant T cells in the irradiated recipients (Kataoka & Sado, 1975) may play an important part in the memory expression is not eliminated. It is also possible that some weakly Thy-1-positive cells surviving after the treatment with anti-Thy-1 antibody plus complement, may co-operate with the memory cells in the expression of the IgM and IgG memory against TI-2 challenge. In a preliminary experiment, both TD antigen-primed memory and TI-2 antigen-primed memory were not recalled by TD challenge under a T cell-depleted condition prepared by the same methods as in the present study (data not shown). Therefore, it seems allowable to conclude that the expression of the B memory by TI-2 challenge is independent of T cell help as compared with the case of B memory expression by TD challenge.

When TD antigens were used for both priming and challenge, T cell help was not required for the induction but was required for the expression of IgG memory (Schrader, 1975; Okumura *et al.*, 1976). In clear contrast with this, the present results have demonstrated that the induction but not the expression of IgG memory to TI-2 antigen is dependent on the presence of T cells. Furthermore, IgM memory cells to DNP-DE were elicited in athymic nude mice (Table 1). Therefore, the class switch from IgM to IgG in the memory B cell pool to TI-2 antigens seems dependent on some kind of T cell help. Some years ago, Andersson & Blomgren (1975) reported that antibody responses of B cells from young mice against a TI-2 antigen are dependent on the presence of T cells. Moreover, it has recently been reported that IgG antibody production elicited by TI-2 antigens is under T cell control (Mongini, Stein & Paul, 1981). Thus, there are some B cells which require T cell help when they are activated by TI-2 antigens to generate AFC or memory cells. What kind of T cells, carrier-specific helper, hapten-specific helper or amplifier, are involved in the induction of IgG memory cells by TI-2 priming is one of the interesting questions to be answered.

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REFERENCES

ANDERSSON B. & BLOMGREN H. (1975) T-cell response to polyvinyl pyrrolidone is linked to maturity of B cells. *Nature (Lond.)*, **253**, 476.

BAKER P.J., STASHAK, P.W., AMSBAUGH D.F. & PRESCOTT B. (1971) Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. I. Dose-response studies and the effect of prior immunization on the magnitude of the antibody response. *Immunology*, **20**, 469.

CLAMAN H.N., CHAPERON E.A. & TRIPLETT R.F. (1966) Immunocompetence of transferred thymus-marrow cell combinations. *J. Immunol.* **97**, 828.

COLLE J., MOTTA I. & TRUFFA-BACHI P. (1983) Generation of immune memory by haptenated derivatives of thymus-independent antigens in C57BL/6 mice. I. The differentiation of memory B lymphocytes into antibody-secreting cells depends on the nature of the thymus-independent carrier used for memory induction and/or revelation. *Cell. Immunol.* **75**, 52.

DIAZ-ESPADA F., MARTINEZ-ALONSO C. & BERNABE R.R. (1978) Development of B cell subsets: effect of priming on *in vitro* TNP-LPS responsiveness. *J. Immunol.* **121**, 13.

EISEN H.N., KERN M., NEWTON W.T. & HELMREICH E. (1959) A study of the distribution of 2,4-dinitrobenzene sensitizers between isolated lymph node cells and extracellular medium in relation to induction of contact skin sensitivity. *J. exp. Med.* **110**, 187.

GUERCIO P.D., THOBIE N. & POIRIER M.F. (1974) IgM anamnestic immune response to haptenic determinant DNP on a thymus independent carrier. *J. Immunol.* **112**, 427.

HOSOKAWA T. (1979) Studies on B-cell memory. II. T-cell independent antigen can induce B-cell memory. *Immunology*, **38**, 291.

HOSOKAWA T., AMAGAI T. & MURAMATSU S. (1979) Studies on B-cell memory. I. Generation and exhaustion of B-cell memory by thymus-dependent antigen in T-cell depleted mice. *Immunology*, **38**, 283.

HOSONO M. & MURAMATSU S. (1972) Use of 2-mercaptoethanol for distinguishing between IgM and IgG antibody-producing cells of mice immunized with bovine-γ globulin. *J. Immunol.* **109**, 857.

KATAOKA Y. & SADO T. (1975) The radiosensitivity of T and B lymphocytes in mice. *Immunology*, **29**, 121.

MITCHELL G.F. & MILLER J.F.A.P. (1968) Immunological activity of thymus and thoracic-duct lymphocytes. *Proc. natn. Acad. Sci. U.S.A.* **59**, 296.

MONGINI P.K.A., STEIN K.E. & PAUL W.E. (1981) T cell regulation of IgG subclass antibody production in response to T-independent antigens. *J. exp. Med.* **153**, 1.

OKUMURA K., METZLER C.M., TSU T.T., HERZENBERG L.A. & HERZENBERG L.A. (1976) Two stages of B-cell memory development with different T-cell requirements. *J. exp. Med.* **144**, 345.

RITTENBERG M.B. & PRATT K.L. (1969) Anti-trinitrophenyl (TNP) plaque assay. Primary response of Balb/c mice to soluble and particulate immunogen. *Proc. Soc. exp. Biol. Med.* **132**, 575.

ROELANTS G.E. & ASKONAS B.A. (1972) Immunological B memory in thymus deprived mice. *Nature (Lond.)*, **239**, 63.

SCHOTT C.F. & MERCHANT B. (1979) Carrier-specific immune memory to a thymus-independent antigen in congenitally athymic mice. *J. Immunol.* **122,** 1710.

SCHRADER J.W. (1975) The role of T cells in IgG production: Thymus-dependent antigens induce B cell memory in the absence of T cells. *J. Immunol.* **114,** 1665.

SHARON R., MCMASTER P.R.B., KASK A.M., OWENS J.D. & PAUL W.E. (1975) DNP-lys-Ficoll: a T-independent antigen which elicits both IgM and IgG anti-DNP antibody-secreting cells. *J. Immunol.* **112,** 427.

UMETSU D.T., CHAPMAN-ALEXANDER J.M. & THORBECKE G.J. (1979) Cross-priming of murine B cells with TNP conjugates of hemocyanin and Ficoll: Characteristics of primed B cells responding to both antigens. *J. Immunol.* **123,** 396.