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# *Studies on the Influence of T Cells in Antibody Production**

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Clear-cut evidence is now available for the involvement of at least two lymphoid cell types in antibody responses to some heterologous erythrocytes and serum proteins (reviewed in *Transplant. Rev.*, 1969, Vol. 1). Studies on immunological tolerance (Chiller *et al.*, 1970; 1971.) and carrier–hapten relationships (Raff, 1970a) strongly suggest that antigenic reactivity is a property of both cell types. At the present time, antibody secretion has been demonstrated only in cells derived directly, or after a period of maturation (in a site other than the thymus), from cells in the bone marrow. The site of origin of these cells (B cells) is presumably homologous to the bursa of Fabricius in chickens

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(Warner, 1967; Peterson *et al.*, 1966). Thymus-influenced cells (T cells, thymus-dependent cells, thymus-derived cells) facilitate total antibody production in the B-cell lineage at least to the antigens mentioned above.

To account for the interaction between T and B cells, several hypotheses have appeared in the literature. Those proposed by Mitchison (1969), Burnet (1968), and Bretscher and Cohn (1970) take into consideration the property of antigenic reactivity in both cell types. T cells either promote triggering of B cells by means of judicious antigen presentation (Mitchison, 1969; Bretscher and Cohn, 1970) or elaborate a substance which does not have immunological specificity and which influences antibody production by the B-cell lineage in any number of conceivable ways (Burnet, 1968).

In our attempts to provide information on the nature of the interaction between T and B cells, we have sought answers to several questions: (a) Is specific long-term immunological memory carried by one or the other or both cell types? (b) What is the predominant class, affinity, and degree of heterogeneity of the antibodies present in thymectomized mice injected with various antigens? (c) Does the reduced response of thymectomized mice to sheep erythrocytes (SRBC) reflect the activity of a residual population of T cells or the activity of B cells responding in the absence of T cells? (d) Can we find evidence for a factor elaborated by antigen-stimulated T cells which facilitates *in vivo* antibody production in the B-cell line?

## I. The Theta ($\theta$) Alloantigen as a Marker for T Cells

The $\theta$ alloantigen of the mouse is expressed maximally in brain and thymus (Reif and Allen, 1964; Aoki *et al.*, 1969). Athymic and surgically thymectomized mice contain in their lymph nodes and spleen a reduced number of $\theta$-bearing cells (Raff, 1970b) and cells sensitive to cytotoxic anti-$\theta$ sera (Schlesinger and Yron, 1970; Raff and Wortis, 1970). These observations suggest that $\theta$ may be a surface antigen of T cells distinguishing these lymphocytes from non T cells in the peripheral lymphoid organs. Concerning the effect of thymectomy on the number of $\theta$-bearing cells in peripheral sites, we have confirmed the observations of others using AKR anti-$\theta^{C3H}$ serum (anti-$\theta$) and a fluoresceinated anti-AKR allotype serum (Table I).

The mouse strain used in this study was C57BL/10, b/b, p/p, a strain designated BCT in these laboratories and which carries the alleles $\theta^{C3H}$ at the $\theta$ locus and b at the immunoglobulin allotype locus (Herzenberg

*et al.*, 1968). The fluoresceinated antiserum was an anti-a allotype serum raised in C57BL/10 (BCT) mice which cross-reacts with AKR serum (d allotype). The two pools of anti-$\theta$ used gave slightly different proportions of fluorescence-positive cells in lymphoid organs. Nevertheless, it is clear from the data in Table I that there is a ranking of fluorescence positivity from thymus–thoracic duct–lymph nodes and Peyer's patches and that thymectomized radiation chimeras contain relatively fewer fluorescence-positive cells in their lymph nodes. The distribution of fluores-

TABLE I

PERCENT FLUORESCENCE-POSITIVE CELLS IN LYMPH NODES FROM THYMECTOMIZED AND CONTROL MICE USING AKR ANTI-$\theta^{C3H}$ AND FLUORESCEINATED ANTI-AKR ALLOTYPE SERA[a]

| Donors of pooled Cells | Anti-$\theta^{C3H}$ Serum[b] | Percent positive cells in: | | | |
|---|---|---|---|---|---|
| | | Thymus | Lymph nodes | Thoracic duct | Peyer's patches |
| Intact C57BL/10 (BCT) | Pool 1 | 100, 100 | 34, 39, 39 | — | 22 |
| | Pool 2 | 100, 100 | 36, 49, 53, 59 | 76, 70 | 51, 59 |
| Sham thymectomized[c] C57BL/10 (BCT) | Pool 1 | — | 38 | — | — |
| | Pool 2 | — | 51 | — | — |
| Thymectomized[c] C57BL/10 (BCT) | Pool 1 | — | 13 | — | — |
| | Pool 2 | — | 22 | — | — |

[a] Anti-AKR allotype serum is an anti-a allotype serum raised in C57BL/10 (BCT) mice (b allotype), fluoresceinated by the FITC method, and absorbed with C57BL/10 (BCT) lymphoid cells. Data of Masuda, Mitchell, and Herzenberg.

[b] Pool 1 is an AKR/J anti-C3H/HeJ thymus and mesenteric lymph node serum absorbed with C57BL/10 (BCT) serum on sepharose. Pool 2 is an AKR/J anti-C3H/HeJ thymus serum.

[c] Neonatal or adult operated mice, both types irradiated with 700 rad and injected with five million syngeneic marrow cells.

cence was patchy in cells of all organs examined, and no differences in staining pattern were apparent between fluorescence-positive cells of thymectomized and sham-thymectomized mice.

If these particular anti-$\theta$ sera detect only T cells and if the majority of thoracic duct cells are T cells, then the above results are surprising in view of the much more striking differences in output of thoracic duct cells in thymectomized and sham-thymectomized mice (Miller and

Mitchell, 1969). Perhaps many of the T cells are recruited by antigen (Sprent *et al.*, 1971) out of the circulating pool in thymectomized mice and/or are relatively sessile cells in peripheral lymphoid organs. By extrapolation from the work of Ford and Gowans (1967) and Strober (1970) in rats, noncirculating T cells may be far less effective at initiating some *in vivo* antibody responses on the part of B cells. Thus, the impaired immunological reactivity of thymectomized mice to some antigens may reflect a marked reduction in *circulating* T cells rather than *total T cell number* in peripheral lymphoid organs.

### II. Influence of Treatment with Anti-$\theta$ on the *in vitro* Response of Spleen Cells from Neonatally Thymectomized Mice to SRBC

The presence of numerous cells in the tissues of thymectomized mice reacting with anti-$\theta$ raises the possibility that the reduced response of such mice to SRBC may be further reduced by treatment with anti-$\theta$. Spleen cells from four neonatally thymectomized CWB/8 mice ($\theta^{C3H}$, $Ig^b$) were treated at 37°C with anti-$\theta$ or normal AKR mouse serum (NMS) together with guinea pig serum (GPS) according to the method previously described (Chan *et al.*, 1970). After incubation, the cells were washed, counted, and cultured with SRBC in Mishell–Dutton cultures (Mishell and Dutton, 1967). Numbers of direct plaque-forming cells (PFC) were determined 5 days later, and the results are presented in Table II. Spleen cells from three of the four mice responded to SRBC by producing PFC *in vitro*. However, these low responses were reduced substantially by treatment with anti-$\theta$ + GPS. In this system we have shown that *in vivo* "educated" thymus cells (Mitchell and Miller, 1968) restore the responses of normal spleen cells treated with anti-$\theta$ + GPS (Chan *et al.*, 1970). The finding suggests that B cells *in vitro* may not respond to SRBC by producing direct PFC in the absence of T cells.

### III. Influence of Thymectomy on IgM and IgG Antibody Production to SRBC and the Synthetic Polypeptide (T,G)-A–L

Miller *et al.* (1967) and Taylor and Wortis (1968) have shown that both IgM (19S) and IgG (7S) antibody responses to SRBC are reduced in thymectomized mice. However, at the level of both serum antibody

**TABLE II**

INHIBITION OF *in vitro* RESPONSE OF SPLEEN CELLS FROM NEONATALLY THYMECTOMIZED MICE USING ANTI-$\theta$ + GPS[a]

| Mouse no. | Treatment *in vitro* | Direct PFC per $10^6$ recovered cells on day 5 of culture[b] |
|---|---|---|
| 1 | NMS + GPS | 5 |
| | Anti-$\theta$ + GPS | 3 |
| 2 | NMS + GPS | 113 |
| | Anti-$\theta$ + GPS | 9 |
| 3 | NMS + GPS | 16 |
| | Anti-$\theta$ + GPS | 1 |
| 4 | NMS + GPS | 77 |
| | Anti-$\theta$ + GPS | 16 |

[a] Neonatally thymectomized CWB/8 mice, 5–7 weeks old. Data of Chan, Mitchell, and Mishell (unpublished).

[b] Mishell–Dutton culture with SRBC (Mishell and Dutton, 1967).

and PFC production, IgG responses are reduced more strikingly than IgM responses. This is apparent also in the limited data presented in Table III in which the 8-day indirect PFC response in neonatally thymectomized mice is 3 $\log_{10}$ units lower than in sham thymectomized litter mates.

The difference in susceptibility of IgM versus IgG antibody responses to thymectomy is particularly apparent in the genetically controlled, H-2 linked response of mice to (T,G)-A–L (McDevitt and Benacerraf,

**TABLE III**

PFC RESPONSES IN 5- TO 6-WEEK-OLD NEONATALLY THYMECTOMIZED CWB/8 MICE INJECTED WITH $10^7$ SRBC

| Operation at birth | No. of mice | PFC per spleen at 8 days[a] | |
|---|---|---|---|
| | | Direct | Indirect |
| Thymectomy | 10 | 780 (540–1120) | 30 (10–90) |
| Sham thymectomy | 5 | 6830 (4510–10,340) | 72,250 (52,510–99,420) |

[a] Results expressed as geometric mean and limits of standard error in brackets. Indirect PFC developed with a polyvalent rabbit anti-mouse $\gamma$-globulin serum (data of Mitchell and Herzenberg).

1969). Grumet (1971) recently demonstrated that antigen-binding titers of early IgM antibody do not differ in C3H.SW mice ($H\text{-}2^b$, "responder") and their congenic partner C3H ($H\text{-}2^k$, "nonresponder") injected with aqueous (T,G)-A–L. "Responders" produce 2-mercaptoethanol (2-ME) resistant anti-(T,G)-A–L antibodies after booster injections, whereas "nonresponders" do not. Much indirect evidence suggests a T-cell involvement in the genetic control of this antibody response, and it was of interest to examine the effect of thymectomy on the primary response of C3H.SW and C3H mice to aqueous (T,G)A–L.

Young adult C3H.SW and C3H mice were thymectomized, irradiated (750 rads), and injected with syngeneic bone marrow cells. Four weeks

**TABLE IV**

INFLUENCE OF THYMECTOMY ON PRIMARY AND TERTIARY ANTIBODY RESPONSES TO 10 μg AQUEOUS (T,G)-A–L IN "RESPONDER" (C3H.SW) AND "NONRESPONDER" (C3H) STRAINS OF MICE[a]

| Mice | | Peak percent antigen bound by pooled sera after: | | | |
|---|---|---|---|---|---|
| | | First injection | | Third injection | |
| Strain | Operation | −ME | +ME | −ME | +ME |
| C3H.SW | Thymectomy | 15 | <5 | <5 | |
| C3H.SW | Sham thymectomy | 24 | <5 | 75 | 67 |
| C3H | Thymectomy | 35 | <5 | <5 | |
| C3H | Sham thymectomy | 32 | <5 | <5 | |

[a] Data of Mitchell, Grumet, and McDevitt.

after irradiation, groups of four mice were injected i.p. with 10 μg (T,G)-A–L and again 1 week and 5.5 weeks later. Results of antibody determinations performed on plasma collected after the first and third injections are presented in Table IV. Thymectomized and sham-thymectomized C3H.SW and C3H mice had comparable titers of antigen-binding antibodies after the first antigen injection. This antibody was sensitive to treatment with 2-mercaptoethanol. By contrast, only sham-thymectomized C3H.SW contained binding antibodies (which were insensitive to 2-ME) in the plasma after a third injection of antigen. C3H mice and thymectomized C3H.SW mice did not respond by producing any detectable antibody. This data provides strong support for the notion that *in vivo* IgM antibody responses are less "thymus-dependent" than IgG antibody responses.

## IV. Carriage of Long-Term Memory by Both T Cells and B Cells

Specific immunological tolerance appears to be a property of both B cells (Chiller *et al.*, 1970, 1971; *Transplant. Rev.*, 1969, Vol. 1) and T cells (Smith *et al.*, 1966; Taylor, 1968; Miller and Mitchell, 1970; Many and Schwartz, 1970). If this is the case then it is conceivable that specific immunological memory would also be carried by both cell types. The results of Jacobson *et al.* (1970) clearly indicate that, in a transfer system, memory to SRBC cannot be carried only by the T-cell lineage. In various mixtures of spleen cells from normal or SRBC-immune C3H.SW and CWB congenic mice, all indirect PFC developed with antiallotype sera carried the immunoglobulin allotype marker of the immune spleen cell donor. However, there are a number of experimental results which suggest that T cells behave as a hyperreactive cell population after exposure to erythrocyte or serum protein antigens: These are (a) the results of Cunningham (1969) in an IgM memory system (b) evidence for sensitivity of carrier-reactive cells to anti-$\theta$ serum (Raff, 1970a), (c) evidence for "education" of T cells (Mitchell and Miller, 1968), and antigen-mediated proliferation of T cells (Davies, 1969). The results of the following experiments using anti-$\theta$, congenic C3H.SW, and CWB mice, and specific antiallotype sera indicate that specific immunological memory to heterologous erythrocytes is carried by T and B cells. They also demonstrate that the secondary adoptive response to flagellin is reduced by treatment of spleen cells with anti-$\theta$.

C3H.SW mice were injected with 10 $\mu$g of polymerized flagellin from *Salmonella adelaide* i.p. and/or high dose SRBC and 10–20 $\mu$g Piromen endotoxin. Four to 21 weeks later the mice were killed, the spleens removed, and the cells incubated *in vitro* with anti-$\theta$ + GPS or NMS + GPS. One to five million washed cells were transferred to irradiated recipients together with $4 \times 10^8$ SRBC or 0.5 to 10 $\mu$g polymerized flagellin i.v. PFC assays (at 7 days) and titrations for bacterial immobilizing antibody (at 7 and 14 days) were performed on spleen cells and sera, respectively, using standard techniques (Mishell and Dutton, 1967; Nossal, 1959). In some experiments, one group of irradiated recipients received dissociated thymus cells from normal CWB mice together with anti-$\theta$ + GPS treated spleen cells. The results are presented in Table V and Fig. 1. Spleen cells treated with anti-$\theta$ + GPS resulted in markedly reduced indirect PFC (developed with antiallotype sera) and antiflagellin antibody responses in both antigen systems. Normal thymus cells, however, were able to increase the response to control

## TABLE V

INHIBITION OF ADOPTIVE SECONDARY RESPONSE TO FLAGELLIN USING ANTI-$\theta$ SERUM[a]

| Dose of antigen ($\mu$g) | Treatment *in vitro* | Thymus cell supplement[b] | Serum antibody titers (± SE)[c] 7 days | | 14 days | |
|---|---|---|---|---|---|---|
| | NMS + GPS | — | 6.7 ± 0.7 (12)[a] | P < 0.005 | 8.3 ± 1.6 (6) | P < 0.01 |
| | Anti-$\theta$ + GPS | — | 3.1 ± 0.4 (13) | | 4.0 ± 0.9 (7) | |
| 0.5 | Anti-$\theta$ + GPS | 8 to 10 × $10^7$ | 3.3 ± 0.6 (9) | | 5.0 ± 1.5 (5) | |
| | No cells | 8 to 10 × $10^7$ | <1.0 (7) | | <1.0 (2) | |
| | No cells | — | <1.0 (3) | | | |
| | NMS + GPS | — | 8.1 ± 0.6 (10) | P < 0.005 | 9.3 ± 0.7 (15) | N.S. |
| | Anti-$\theta$ + GPS | — | 4.1 ± 0.8 (7) | | 8.1 ± 0.6 (10) | |
| 5 to 10 | No cells | — | <1.0 (7) | | <1 (6) | |
| | Normal cells[d] | — | <1.0 (6) | | 1.3 ± 0.6 (3) | |

[a] Irradiated recipients were injected with five million nucleated cells from pools of spleens from a total of 8 C3H.SW mice injected 4–14 weeks previously with 10$\gamma$ polymerized flagellin from *Salmonella adelaide* i.p. Data of Mitchell, Chan, and Amkraut.

[b] Thymus cell donors and irradiated recipients (number indicated in brackets) were CWB mice.

[c] Titers determined by technique of Nossal (1959), modified for use in microtiter plates. Starting dilution was 1/10 and numbers indicate the well number showing estimated 95% immobility of *S. adelaide* organisms. P values determined by Student t test.

[d] Five million nucleated cells from a pool of spleens from three normal C3H.SW mice.

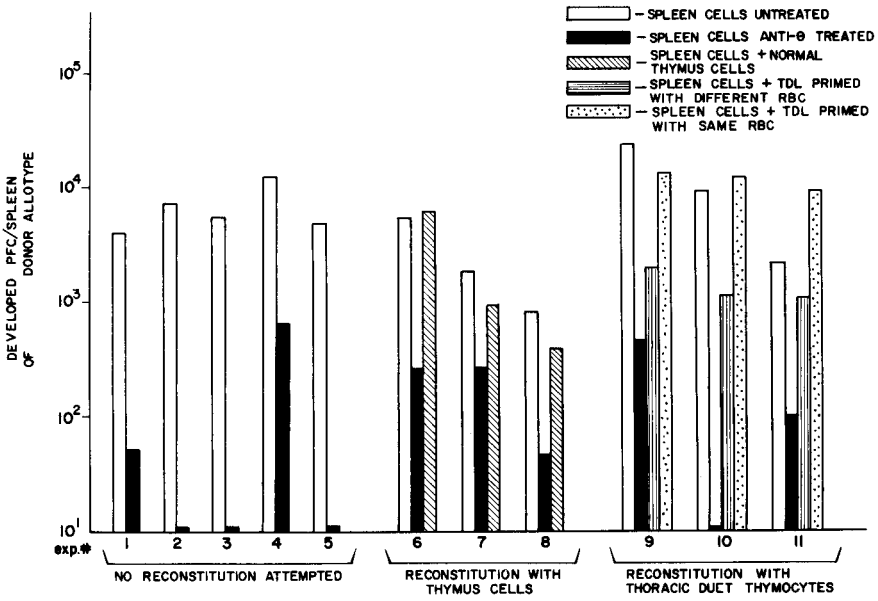


*Fig. 1.*

levels only in the case of the adoptive response to SRBC (Fig. 1, expts. 6–8, cf., Table V). In addition, no differences in serum antiflagellin titer were apparent in recipients of cells treated with NMS + GPS and anti-$\theta$ + GPS 14 days after transfer of cells and a high dose antigen challenge.

In other experiments (Fig. 1, expts. 9–11) spleen cells from mice immunized with HRBC and endotoxin were treated *in vitro* with anti-$\theta$ + GPS or NMS + GPS. Cells treated with anti-$\theta$ + GPS were supplemented with thoracic duct cells (TDC) from mice immunized to either HRBC or SRBC. Consistently higher reconstituted PFC responses were obtained with TDC from mice immunized against the same erythrocyte type (HRBC) as the donor of the anti-$\theta$ + GPS treated spleen cells. Thus, it seems that memory is carried by both T and B cells. B cells are unable to express significant IgG PFC memory in the absence of cells sensitive to anti-$\theta$ + GPS. This latter population can be replaced functionally by thymus cells themselves or another cell population with T cell activity (i.e., TDC). Clearly, expression of B-cell memory does not depend on an inoculum of *sensitized* T cells in this adoptive transfer system.

TABLE VI

PFC RESPONSE IN RECIPIENTS OF A MIXTURE OF SRBC AND HRBC, THORACIC DUCT CELLS, AND SPLEEN CELLS TREATED WITH ANTI-$\theta$ + GPS

| Spleen cells[a] | | Thoracic duct cells | | Irradiated recipients | | | Indirect PFC per spleen developed with RAM$\gamma$G at 7 days |
|---|---|---|---|---|---|---|---|
| | | | | | Antigen | | |
| Donor immune to: | Treatment (+GPS) | Donor immune to: | No. | No. | Injected | Assay | |
| HRBC | NMS | — | — | 3 | H | H | 6,830 |
| | Anti-$\theta$ | — | — | 4 | H | H | 130 |
| | Anti-$\theta$ | S | $2.5 \times 10^6$ | 4 | H | H | 1,300 |
| | Anti-$\theta$ | S | $2.5 \times 10^6$ | 4 | S + H | H | 1,600 |
| HRBC | NMS | — | — | 3 | H | H | 40,870 |
| | Anti-$\theta$ | — | — | 2 | H | H | 1,750 |
| | Anti-$\theta$ | S | $2 \times 10^6$ | 2 | H | H | 5,300 |
| | Anti-$\theta$ | S | $2 \times 10^6$ | 3 | S + H | H | 8,630 |
| SRBC | NMS | — | — | 4 | S | S | 5,080 |
| | Anti-$\theta$ | — | — | 4 | S | S | 120 |
| | Anti-$\theta$ | H | $2 \times 10^6$ | 4 | S | S | 875 |
| | Anti-$\theta$ | H | $2 \times 10^6$ | 2 | H + S | S | 1,500 |

[a] Three million treated cells injected into each irradiated recipient. Donors injected 7–12 weeks previously with $4 \times 10^8$ heterologous erythrocytes plus 10–20 $\mu$g Piromen i.p. Data of Mitchell *et al.* (1971).

It was of interest to determine whether T cells from mice primed to one erythrocyte type would increase PFC production by anti-$\theta$-treated spleen cells from mice primed to the other erythrocyte type. This may occur in irradiated recipients injected with both erythrocyte types. The results of experiments presented in Table VI indicate that we have been unable to find supporting evidence, in this *in vivo* system, for the proposed factor elaborated by antigen-activated T cells *in vitro* (Hartmann, 1970).

## V. Discussion

The results of the various studies reported here do not provide obvious support for any particular proposal on the nature of the interaction between T and B cells in antibody responses. However, we believe that several of the observations must be taken into account in any such proposal. The evidence for specific immunological memory in each of the cell types (Fig. 1) further substantiates contentions that both T and B cells behave as specific antigen-reactive cells. This is no way prejudices the question of whether a factor with no immunological specificity, elaborated by T cells, influences antibody production in the B-cell lineage.

The evidence for B cell memory of Jacobson *et al.* (1970) need not conflict with the evidence for T cell memory of Cunningham (1969). Cunningham (1969) demonstrated that the direct PFC-producing activity of B cells from unprimed mice was increased after combination with low numbers of spleen cells from primed mice. In carrier–hapten systems, antihapten antibody production can be increased by priming with the carrier only (Katz *et al.*, 1970; Kettman and Dutton, 1971). Such antibody responses, however, are markedly increased if the carrier–hapten complex is used, rather than the carrier only, as a priming injection. The results of Jacobson *et al.* (1970) might then be anticipated on the basis of prior hapten sensitization in contrast to the situation in the system of Cunningham (1969) in which hapten-reactive cells (cf., carrier-reactive cells) could be diluted out in the spleen cell inoculum. The present system has provided evidence for memory in both cell populations.

Recent studies (Wang *et al.*, 1970; Kincade *et al.*, 1970) support the claim of a $\mu$-to-$\gamma$ chain "switch" in cells of the B-cell lineage (Nossal *et al.*, 1964). This conversion from $B^{\mu}$ cells to $B^{\gamma}$ cells could occur prior to or after contact and stimulation with antigen. IgM antibody responses are less affected by thymectomy than IgG antibody responses

(Tables III and IV; Miller *et al.* 1967; Taylor and Wortis, 1968). This raises the possibility that T cells influence either directly or indirectly, a conversion of $\mu$-chain expression to $\gamma$-chain expression in B cells. Since thymectomized mice have been reported to contain normal levels of circulating IgG (in Miller and Osoba, 1967), it is unlikely that T cells are obligatory for any $C_H$ gene conversion in B cells. However, data from studies on antibody responses in surgically thymectomized mice must be interpreted with caution. With the assumption that anti-$\theta$ reacts with T cells only, it is quite clear that peripheral lymphoid organs of thymectomized mice contain an abundance of T cells (Table I; Raff, 1970b). Even if this assumption is unwarranted, it is likely that a division of antibody responses into "thymus dependent" and "thymus independent" is meaningless in situations other than those involving animals known to lack an *in utero* epithelial component of the thymus. The low direct PFC *in vitro* response of spleen cells from neonatally thymectomized mice is virtually abolished by treatment with anti-$\theta$ + GPS. Hence, B cells (including potential IgM secretors) may not respond to SRBC by producing antibody-secreting progeny in the absence of T cells, at least *in vitro*.

Again, caution is required in interpreting any data in which inhibition of a response is the consequence of an antiserum treatment. Until reconstitution is achieved, one can only guess at the probable cellular site of action of the antiserum. In this respect antiallotype antibodies and anti-H-2 sera are powerful tools for identifying cell origins in reconstitution systems. The present results (Fig. 1) and those published for an *in vitro* primary response (Chan *et al.*, 1970) indicate that precursors of direct and indirect PFC (B cells) are not affected, as far as can be determined, by treatment with anti-$\theta$ + GPS. In the case of the adoptive secondary response to polymerized flagellin (Table V), no reconstitution could be achieved with thymus cells. This may reflect a low number of antiflagellin reactive cells in the virgin T-cell population or an inhibitory effect of anti-$\theta$ + GPS treatment on some B cells reactive to flagellin. Possibilities will be narrowed and interpretations refined by a demonstration of reconstitution in this system.

Treatment with anti-$\theta$ + GPS does not reduce numbers of direct or indirect PFC themselves (Chan *et al.*, 1970; Schlesinger, 1970; Takahashi *et al.*, 1970), hemopoietic stem cells (Chan *et al.*, 1970; Mitchell *et al.*, 1971) or the precursors of direct and indirect PFC as measured by ultimate adoptive PFC responses after reconstitution (Chan *et al.*, 1970; Fig. 1). In the case of *in vitro* and *in vivo* responses following treatment with anti-$\theta$ + GPS, we have shown that the activity of a fully reconstitutive inoculum is reduced by treatment with anti-$\theta$ + GPS

(Chan *et al.*, 1970; Mitchell *et al.*, 1971). The interpretation seems warranted that in terms of function, cells sensitive to anti-$\theta$ + GPS can be replaced by T cells.

## VI. Summary

Surgical thymectomy and cytotoxic AKR anti-$\theta^{C3H}$ serum (anti-$\theta$) have been used to dissect the cellular events of several primary and secondary antibody responses. The lymph nodes of thymectomized mice contain cells which react with anti-$\theta$ even though the proportion is reduced when compared with lymph nodes cells from sham-thymectomized mice. Thymectomized mice respond to SRBC and (T,G)-A–L by producing substantial numbers of direct PFC or amounts of 2-ME sensitive antibody but few indirect PFC and no detectable 2-ME resistant antibody, respectively. Treatment with anti-$\theta$ + GPS virtually abolishes the primary *in vitro* direct PFC response of spleen cells from thymectomized mice to SRBC. Similarly, the adoptive secondary response of spleen cells from SRBC-immune mice is reduced to very low levels by treatment with anti-$\theta$ + GPS. Thymus and thoracic duct (TDC) both reconstitute indirect PFC responses. TDC from specifically primed mice are better than cells from nonspecifically primed mice at reconstituting an adoptive PFC response of anti-$\theta$ + GPS treated spleen cells from immunized mice.

The data indicate that memory to heterologous erythrocytes can be carried by both T and B cells and that B cells require T cells, but not necessarily primed T cells, for expression of IgG memory.

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