STIMULATION OF IgG ANTIBODY RESPONSE IN VITRO BY T CELL-REPLACING FACTOR*

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Cooperation between different lymphoid cell populations, thymus-derived (T) and non-thymus-derived (B) cells, in the optimal immune response to various antigens has become a well-established fact in recent years (1). Hapten-carrier studies (2, 3) indicate that this cooperation may depend on a close physical contact between the two cell types, each one recognizing specific determinants on an antigenic molecule. The detailed mechanisms behind the synergism have, however, remained relatively unclear. The most popular theories about the interaction between T and B cells include (a) the concentration of antigen on the surface of the T cell and presentation to the B cell (2) and (b) the production of soluble factors by T cells upon contact with their specific antigens, which in turn would stimulate the linked B cell. In the last model, both nonspecific (4-6) and antigen-specific (7) factors have been suggested to play a role. The involvement of an antigen-specific T cell factor would, however, require the participation of a third-party cell, e.g., macrophages (7). Using the Mishell-Dutton in vitro system of mouse lymphoid cells for antibody production against various heterologous red blood cells (8), we have undertaken to study the mechanisms involved.

We have established that in this system the 19S response against sheep red blood cells (SRBC) is highly T cell dependent, since it can be abrogated by treatment of spleen cells with antitheta serum and complement (9). Complete reconstitution of the response can be achieved by antigen-activated T cells (10), by allogeneic T cells (11), and also and most interestingly by a soluble T cell-replacing factor (4) (TRF). TRF can be obtained from spleen cells, lymph node cells, and thymus cells (12) subjected to heavy stimulation by transplantation antigens unrelated, to the best of our knowledge, to the antigens used in the test system, namely sheep, horse, and chicken red blood cells. TRF production has been shown to be dependent on the presence of T cells (4) and to be nonantigen specific also in its stimulatory function by all criteria applied (12). The factor can fully replace T cell function for the antigens used under the most stringent conditions of T cell depletion in spleen cultures from the congenitally athymic nude mice (13), even after treatment with anti-θ-serum and complement. We have therefore suggested that TRF is a physiological product in all normal T-B cell contacts and that the proximity of T and B cells is necessary to assure that TRF can be transferred from the T cell to the B cell (4). Soluble products with similar properties have recently been described by various groups (5, 14).

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In order to support the notion that TRF has some physiological role to play, it would be desirable to show that TRF can stimulate not only IgM (19S) but also IgG (7S) responses, since the latter are even more strikingly dependent on T cell function (15) and, moreover, physiologically more important. This communication shows that an IgG (7S) response to SRBC can be obtained by adding TRF to T cell-deprived spleen cultures of preimmunized mice (12).

Materials and Methods

8-12-wk old B6D2F1 mice (C57BL/6J X DBA/2J)F1 from Jackson Laboratory, Bar Harbor, Maine, were immunized by the intraperitoneal injection of 1 X 10^6 sheep red blood cells in balanced salt solution (BSS) 8, 9, and 10 days before sacrifice. Spleen cell suspensions were then prepared as previously described (8). Removal of T cells, according to Raff (16), was achieved by simultaneous treatment of aliquots each of 3 X 10^7 cells with 0.5 ml of AKR anti-0-C3H ascitic fluid and 0.5 ml of guinea pig complement (final dilutions 1:5, respectively) for 30 min at 37°C. Controls were treated similarly but anti-0-serum was replaced by 0.5 ml of culture medium containing 20% of fetal bovine serum.

After the incubation, the cells were washed twice with BSS in the cold. They were re-suspended in culture medium (8), the cell concentration being adjusted to 1.6 X 10^7 cells per ml. 0.5 ml of this suspension was seeded into small Petri dishes. The antigen dose usually consisted of 5 X 10^6 SRBC per plate. Where required, 0.5 ml of TRF preparation (4) was added immediately, controls receiving 0.5 ml of additional culture medium instead. The plates were then placed on a rocker platform and incubated at 37°C in an atmosphere of 10% CO2, 7% O2, 83% N2. In some experiments, 0.5 ml of medium was withdrawn after the times indicated and 0.5 ml of TRF was freshly added.

Immune responses were usually assayed at day 4 of culture by the hemolytic plaque technique of Jerne as modified by Mishell and Dutton (17, 8). IgM (19S) producers were measured as direct plaque formers with complement alone. For the demonstration of IgG (7S) plaques, parallel slides were incubated for 1 h with BSS and then treated for 45 min with concanavalin A (0.4 mg/ml in BSS) according to Nordin et al. (18). Thereafter, the slides were carefully washed in BSS and the plaques were developed during a 2 h incubation with rabbit antimouse IgG serum diluted 1:100 in 10% guinea pig serum as a source of complement. By this procedure, consistently less than 1% of 19S plaque-forming cells (PFC) were developed when using primary cultures. Plaque-forming cells are given as numbers per 10^6 cells, counted in a Coulter Counter Model F (Coulter Electronics, Inc., Hialeah, Fla.) at the appropriate aperture. The variations between parallel cultures were ±25%. Each culture consisted of two parallel Petri dishes that were pooled and assayed on two parallel slides per dilution.

RESULTS AND DISCUSSION

Table I gives the results obtained after culturing spleen cells from SRBC-primed mice. As has been shown by Chan et al. (10), anti-0 treatment of cells at this stage of immunization (~8, ~9, or ~10 days) is highly effective in abolishing both the 19S and 7S immune responses. Indeed, though T cell removal from spleen cell suspensions of normal mice may occasionally result in a residual 19S activity of up to 30 or 50% of untreated controls, identical treatment at the same cell and antiserum concentrations consistently reduces the response much more drastically when using cells from primed mice. After the times of priming used, the IgM response seems just as sensitive to T cell de-
pletion as the IgG response. Both responses can, however, be reconstituted with TRF. But there are three important differences to the effects observed in primary cultures. While in primary cultures TRF acts optimally if added at day 2 (4), for the stimulation of a secondary response it has to be present from the onset. This is shown in Tables I and II. In addition, the reconstitution of a

### TABLE I

| Time of priming | Treatment of spleen cells | Supplement day 0 | PFC/10^6 recovered cells |
|-----------------|---------------------------|------------------|--------------------------|
| Exp. I          | GPC MEM                   | 7,250 (41)*      | 2,330 (110)*             |
| Exp. II         | Anti-γ GPC MEM            | 440              | 160                      |
| Exp. III        | Anti-γ GPC Condit. MEM    | 310              | 180                      |
|                 | Anti-γ GPC TRF            | 5,600            | 1,130                     |
| Exp. I          | GPC MEM                   | 10,000 (90)*     | 7,150 (330)*             |
| Exp. II         | Anti-γ GPC MEM            | 140              | 120                      |
| Exp. III        | Anti-γ GPC Condit. MEM    | 150              | 130                      |
|                 | Anti-γ GPC TRF            | 3,220            | 3,050                     |
| Exp. I          | GPC MEM                   | 5,150 (140)*     | 5,700 (200)*             |
| Exp. II         | Anti-γ GPC MEM            | 30               | 60                       |
| Exp. III        | Anti-γ GPC Condit. MEM    | 30               | 110                      |
|                 | Anti-γ GPC TRF            | 1,480            | 1,780                     |

* Imnunization see text.

Condit. MEM, culture medium (5) in which normal B6B2F1 spleen cells (1 × 10^7/ml) were grown for 24 h. TRF was added at day 0 of culture.

GPC, guinea pig serum as a source of complement.

* Figures in parentheses: PFC/10^6 nucleated cells on day 0 of culture.

### TABLE II

| Treatment of cells* | Supplement | Time of addition of supplement to cultures | PFC/10^6 recovered cells |
|---------------------|------------|---------------------------------------------|--------------------------|
|                     | GPC MEM    | 0                                          | 5,300                    |
| Anti-γ + GPC MEM    | 0          | 160                                         |
| Anti-γ + GPC TRF    | 0, 24, 48, 72 | 2,450                                      | 1,820                    |
|                     | 48         | 590                                         | 280                      |

* Spleen cells from B6D2F1 mice were put into culture 9 days after i.p. injection of 5 × 10^7 SRBC. (Day 0: 50 19S PFC/10^6, 60 7S PFC/10^6.) When more than one dose of 0.5 ml of TRF was added, 0.5 ml of culture medium was withdrawn in order to maintain a constant cell concentration.

In summary, we have shown that the T cell-replacing factor obtained from

secondary response by TRF seemingly needs much more of the factor than is required by a primary culture where one dose of TRF often results in a significant overshoot of the response well over control values (Table III). In contrast, even when TRF is added daily, the values for both the IgM and IgG producers in secondary cultures remain lower than those observed in controls (Table II).

In summary, we have shown that the T cell-replacing factor obtained from
mixtures of allogeneic spleen cells is able to reconstitute in vitro both the IgM and the IgG immune responses against SRBC in T cell-deprived spleen cell cultures from primed mice. This fact seems to eliminate one of the arguments most often used against a possible physiological role of the nonspecific soluble product, namely that it may merely enhance a normal thymus-independent response against some components of heterologous blood cells. A 7S stimulation by the allogeneic effect has previously been observed in vivo by Katz et al. (19). Nonspecific B cell mitogens, e.g. lipopolysaccharide, however, failed to lead to a significant 7S response, though it did stimulate a 19S response in T-deprived cultures (20). A greater amount of TRF and a proper timing with respect to its addition seem to be mandatory both for the secondary 19S and 7S responses. This fits well to the observation that TRF is not absorbed by virgin spleen cells but is absorbed or used up after incubation with primed cells (12). All this is most easily reconciled with the assumption that the target cell for TRF is an "intermediary" or "early memory" cell that has already made contact with the antigen and has undergone changes, e.g. capping (21), cell division, etc., which make it receptive to the action of TRF. Under our conditions and with the antigens so far used by us (blood cells of sheep, horse, and chicken), this primary contact seems to take place even in the absence of T cells or antigen-specific products of T cells, though this may be different for other antigens. The B cell would thus reach a stage where contact with a T cell or TRF becomes mandatory for its final development into an antibody-producing cell. TRF might well turn out to be a differentiation factor. This concept seems to be supported by the recent finding that a B memory can be induced in thymectomized, irradiated, bone marrow-protected mice, in spite of the seemingly total absence of antibody formation (22).

In a primary culture it takes about 2 days for the B cell to reach the responsive stage, while spleens from immunized mice already contain antigen-triggered B cells. In addition, it is reasonable to assume that the number of TRF receptive and -absorbing B cells in spleen cultures from preimmunized animal

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TABLE III

| Treatment of spleen cells | Supplement day 2 | PFC/10^6 Recovered cells |
|--------------------------|------------------|-------------------------|
|                          | Day 4 | Day 5 |
| Exp. I GPC               | MEM   | 680  | 2,620 |
| Anti-θ GPC               | MEM   | 70   | 210   |
| Anti-θ GPC               | TRF   | 1,580| 9,750 |
| Exp. II GPC              | MEM   | 1,780|       |
| Anti-θ GPC               | MEM   | 150  |       |
| Anti-θ GPC               | TRF (a) | 37,800 |       |
| Anti-θ GPC               | TRF (b) | 14,600 |       |

TRF (a) and (b) are two different batches.
greatly exceeds that in primary cultures. This then would easily account for the increased amount of TRF required to stimulate in vitro both the 19S and 7S responses in secondary cultures.

**SUMMARY**

A soluble factor (TRF) produced by mixtures of allogeneic mouse spleen, lymph node, and thymus cells functionally replaces T cells in a primary IgM antibody response to sheep blood cells in vitro. It is now shown that TRF can also reconstitute an IgG antibody response in T cell-deprived spleen cultures derived from preimmunized mice. The optimal time of addition and the amount of TRF required differ between primary and secondary in vitro systems.

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**REFERENCES**

1. Miller, J. F. A. P. 1971. Interaction between thymus-dependent (T) cells and bone marrow-derived (B) cells in antibody responses. In Cell Interactions and Receptor Antibodies in Immune Responses. O. Mäkelä, A. Cross, and T. U. Kosumé, editors. Academic Press, New York. 293.

2. Mitchison, N. A., K. Rajewsky and R. B. Taylor. 1970. Cooperation of antigenic determinants and of cells in the induction of antibodies. In Developmental Aspects of Antibody Formation and Structure. J. Sterzl and I. Riha, editors. Academia, Prague. 2:547.

3. Mitchison, N. A. 1969. A reassessment of mechanisms of the immune response. In Immunological Tolerance. M. Landy and W. Braun, editors. Academic Press, New York. 113.

4. Schimpl, A., and E. Wecker. 1972. Replacement of T-cell function by a T-cell product. Nature (Lond.). 237:15.

5. Dutton, R. W., R. Falkoff, J. A. Hirst, M. Hoffmann, J. W. Kappler, J. R. Kettman, J. F. Lesley, and D. Vann. 1971. Is there evidence for a non-antigen specific diffusable chemical mediator from the thymus-derived cell in the initiation of the immune response? In Progress in Immunology. B. Amos, editor. Academic Press, New York. 355.

6. Feldmann, M., and A. Basten. 1972. Specific collaboration between T and B lymphocytes across a cell impermeable membrane in vitro. Nat. New Biol. 237:13.

7. Feldmann, M., and A. Basten. 1972. Cell interactions in the immune response in vitro. IV. Comparison of the effects of antigen-specific and allogeneic thymus-derived cell factors. J. Exp. Med. 136:722.

8. Mishell, R. J., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126:413.

9. Schimpl, A., and E. Wecker. 1970. Inhibition of in vitro immune response by treatment of spleen cell suspensions with anti-θ-serum. Nature (Lond.). 226:1258.

10. Chan, E. L., R. I. Mishell, and G. F. Mitchell. 1970. Cell interaction in an immune response in vitro: requirement for theta-carrying cells. Science (Wash. D.C.). 170:1215.
11. Schimpl, A., and E. Wecker. 1971. Reconstitution of a thymus cell-deprived immune system by syngeneic and allogeneic thymocytes in vitro. *Eur. J. Immunol.* 1:304.

12. Schimpl, A., and E. Wecker. 1972. Functional replacement of co-operating T-cells by a soluble factor in a humoral immune response in vitro. *Adv. Exp. Med. Biol.* 29. In press.

13. Pantelouris, A. M. 1968. Absence of thymus in a mouse mutant. *Nature (Lond.)* 217:370.

14. Ekphah-Mensah, A., and J. C. Kennedy. 1971. New indicator of histocompatibility differences in vitro. *Nat. New Biol.* 233:174.

15. Taylor, R. B., and H. H. Wortis. 1968. Thymus dependance of antibody response: variation with dose of antigen and class of antibody. *Nature (Lond.)* 220:927.

16. Raff, M. C. 1969. Theta isoantigen as a marker of thymus-derived lymphocytes in mice. *Nature (Lond.)* 224:378.

17. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody producing cells. *Science (Wash. D.C.)* 140:405.

18. Nordin, A. A., H. Cosenza, and W. Hopkins. 1969. The use of concanavalin A for distinguishing IgM from IgG antibody-producing cells. *J. Immunol.* 103:839.

19. Katz, D. H., W. Paul, E. A. Goedl, and B. Benacerraf. 1971. Carrier functions in antihapten antibody responses. III. Stimulation of antibody synthesis and facilitation of hapten-specific secondary antibody responses by graft-versus-host reactions. *J. Exp. Med.* 133:169.

20. Sjöberg, O., J. Anderson, and G. Möller. 1972. Lipopolysaccharide can substitute for helper cells in the antibody response in vitro. *Eur. J. Immunol.* 2:326.

21. Taylor, R. B., W. P. H. Duffus, M. C. Raff, and S. de Petris. 1971. Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nat. New Biol.* 233:225.

22. Roelants, G. E., and B. A. Askonas. 1972. Immunological B memory in thymus-deprived mice. *Nat. New Biol.* 239:263.