MECHANISM OF T-CELL HELP IN THE IMMUNE RESPONSE TO SOLUBLE PROTEIN ANTIGENS

II. Reconstitution of Primary and Secondary In Vitro Immune Responses to Dinitrophenyl-Carrier Conjugates by T-Cell-Replacing Factor*

By TH. HÜNIG, A. SCHIMPL, AND E. WECKER

(From the Institut für Virologie und Immunbiologie der Universität Würzburg, 8700 Würzburg, West Germany)

In the preceding paper (1), we have demonstrated the in situ generation and action of a nonantigen-specific mediator in the anamnestic immune response to dinitrophenyl keyhole limpet hemocyanin (DNPKLH). The results obtained showed that in spleen cell cultures from DNPKLH primed and boosted mice (2), carrier-specific helper cells can be activated by KLH to produce a nonantigen-specific soluble mediator that can help the primed B cells in the same culture to mature into anti-DNP PFC-secreting IgG, if these B cells are simultaneously stimulated by DNP on a different carrier. Under these conditions, enough of the nonspecific helper factor seems to be produced to circumvent the necessity for an antigen-bridge between T and B cell.

In the present report this nonantigen-specific mediator produced by KLH-activated T cells and the T-cell-replacing factor (TRF) produced upon allogeneic (3) or concanavalin A (Con A) stimulation (4) are compared in an assay system originally described for the demonstration of TRF activity. In addition, the reconstitution by TRF of primary and secondary immune responses to DNP-protein conjugates will be shown.

Materials and Methods

Mice. DBA/2, C57BL, BALB/c, and nu/nu BALB/c mice were obtained from Bomholtgard, Ry, Denmark. Mice of both sexes were used between 6 wk and 6 mo of age.

Antigens, Immunizations, Tissue Culture, Depletion of T Cells, and Assay for Anti-DNP-PFC. All the above were as described in the preceding paper (1). In the primary in vitro anti-DNP response to DNPKLH, background plaque-forming cells (PFC) to unhaptenated horse red blood cells (HRBC) were also assayed and subtracted from the values obtained with TNPHRBC. These background PFC were negligible in the evaluation of the anamnestic response to DNPKLH. Anti-sheep red blood cells (SRBC) PFC were assayed using the Jerne technique modified as in reference 5.

Preparation and Partial Purification of TRF. TRF derived from allogeneic supernates was

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1 Abbreviations used in this paper: AEF, allogeneic effect factor; Con A, concanavalin A; DNP, dinitrophenyl; HRBC, horse red blood cells; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cells; TRF, T-cell-replacing factor.

1228 THE JOURNAL OF EXPERIMENTAL MEDICINE • VOLUME 145, 1977
TH. HÜNIG, A. SCHIMPL, AND E. WECKER

TABLE I
Reconstitution of the Primary Response to SRBC in Nude Spleen Cell Cultures by a Supernate from DNPKLH-Stimulated Cultures

| Cells and antigen in culture | Addition             | Anti-SRBC PFC/10^6 |
|-----------------------------|----------------------|---------------------|
| nu/nu + SRBC                | 0.5 ml MEM/day 2     | 50                  |
| nu/nu + SRBC                | 0.5 ml TRF-allo/day 2| 924                 |
| nu/nu + SRBC                | 0.5 ml TRF-DNPKLH/day 0| 76          |
| nu/nu + SRBC                | 0.5 ml TRF-DNPKLH/day 2| 837          |

Spleen cell cultures from nu/nu BALB/c mice were set up in half the normal volume in the presence of 5 x 10^6 SRBC. At the times indicated, TRF-allo (3) or "TRF-DNPKLH," a 24-h supernate of spleen cells from DNPKLH primed and boosted mice, stimulated with 0.1 μg DNPKLH/ml, were added. Direct anti-SRBC PFC were assayed on day 5 of culture.

Prepared as described (3). TRF derived from Con A (Pharmacia Fine Chemicals, Uppsala, Sweden)-stimulated spleen cells (4) was partially purified on a Sephadex G-75 (Pharmacia Fine Chemicals) column (6) and concentrated 20-fold using an Amicon eluate concentrator (Amicon N.V., Oosterhout, Netherlands). α-methyl mannoside (Roth KG, Karlsruhe, W. Germany) was added to assay cultures in a final concentration of 10 mg/ml to block possibly remaining Con A that might not have been retained on the Sephadex G-75 column.

Results
In SRBC-stimulated cultures from congenitally athymic nude mice, TRF produced upon allogeneic stimulation reconstitutes the primary immune response best when it is added on day 2 of culture, but has much less effect when added at the beginning (3, 7). This characteristic feature has been used to define TRF and has also been found with TRF induced by Con A (4) and with purified preparations of TRF (Hübner, Müller, Schimpl, and Wecker, to be published). The nonspecific helper factor which is generated and acts in situ during the anamnestic immune response to DNPKLH in vitro (1) was now tested for TRF activity as defined above. Table I shows an experiment in which a 24-h supernate of a DNPKLH-stimulated spleen cell culture from mice primed and boosted with DNPKLH was tested for TRF activity in an anti-SRBC response of nude spleen cell cultures. Indeed, this supernate stimulated the generation of antibody-forming cells to SRBC and, just as TRF, was clearly more effective when added on day 2 of culture than when added at the beginning.

This finding suggests that the same biologic activity is operative in both systems. If this should be the case, the inverse experiment, i.e., the reconstitution by TRF of the in vitro immune response to DNP-protein conjugates in spleen cell cultures lacking T cells should also be possible. Two experimental conditions were chosen. In the first, it was investigated whether TRF derived from allogeneic spleen cell cultures (3) can replace T cells in a primary immune response to DNPKLH in vitro (Fig. 1). Spleen cell cultures from nude mice were set up in half of the normal volume in the presence (group A) or absence (group B) of 5% fetal calf serum and stimulated with DNPKLH. After 48 h, an equal volume of TRF was added and the number of direct anti-DNP PFC tested on day 4 of culture. While DNPKLH alone rather suppressed than enhanced the
Fig. 1. Stimulation of the primary immune response to DNPKLH in nude spleen cell cultures by TRF. Spleen cell cultures from nu/nu BALB/c mice were set up in half the normal volume, in the presence (group A) or in the absence of fetal calf serum (FCS) (group B). After 48 h, cultures received an equal volume of TRF from an allogeneic supernate (3) or medium, and 5% FCS was added to cultures of group B. DNPKLH: 0.3 μg/culture. Direct anti-DNP PFC were assayed on day 4 of culture. Bars represent mean values of three cultures ± standard error.

background of anti-DNP PFC, the combined addition of TRF and DNPKLH resulted in a significant although weak primary immune response to the DNP determinant, particularly in group B. In group A, which had received FCS from the beginning, the absolute number of anti-DNP PFC was markedly higher. Owing to increased background values, however, the difference between cultures with and without TRF was less pronounced. At any rate, a significant primary immune response to DNPKLH can be obtained in spleen cell cultures free of functional T cells and restored with TRF under appropriate culture conditions.

In the second assay system, TRF prepared from Con A-stimulated BALB/c spleen cell cultures (4) was partially purified on a Sephadex G-75 column (6) and concentrated 20-fold by ultrafiltration. The fraction that was most active in a SRBC-stimulated nude spleen cell system as described above was tested in T-cell-depleted spleen cell cultures from mice primed and boosted with DNPKLH. Fig. 2 shows that the addition of this factor to anti-Thy 1.2 plus complement-treated cells resulted in a distinct and dose-dependent reconstitution of the IgG response to DNPKLH.

Since TRF is defined as a nonantigen-specific mediator (3), it should reconstitute the anti-hapten response even when the DNP determinant is offered to the B cells on a carrier different from that used for priming and boosting. Table II
Fig. 2. Reconstitution of the anamnestic response to DNPKLH by TRF. Spleen cells from C57BL mice primed and boosted with DNPKLH were treated with complement (C') or anti-Thy 1.2 + C' and stimulated with 0.1 μg DNPKLH/culture. TRF was prepared by Con A stimulation of BALB/c spleen cells (4) and partially purified on Sephadex G-75 (see Materials and Methods).

shows an experiment in which anti-Thy 1.2 plus complement-treated spleen cells from mice primed and boosted with DNPKLH were stimulated with DNPKLH or DNPBGG. As expected, neither antigen induced more than background IgG-PFC to DNP in the absence of TRF; the addition of 40 μl of partially purified TRF, however, resulted in an equally good response to both conjugates that was as high as that against DNPKLH with KLH-specific helper cells. The addition of TRF in the absence of antigen did not increase the number of anti-DNP IgG PFC. Similar results were obtained using a TRF preparation derived from allogeneic supernates (data not shown). Thus, even when possibly remaining KLH-specific helper T cells were excluded from the reaction by the use of a different DNP-carrier conjugate in vitro than was used for priming and boosting in vivo, TRF restored the anti-DNP IgG immune response of T-cell-deprived spleen cell cultures.

Discussion

The experimental system in which the concept of TRF (3, 8) or similar nonantigen-specific T-cell helper factors (9-14) has been developed is the in vitro immune response of mouse spleen cells to heterologous erythrocytes. In this system, helper T cells in both the primary (3) and in the secondary (15)
responses can be replaced by the addition of TRF produced upon allogeneic (3) or mitogenic (4) stimulation of T cells. The results obtained in the immune response to red blood cells might, however, not be representative for the general mechanism of T/B cooperation, but might rather be a phenomenon restricted to particulate antigens. Several reports in the literature describe the failure to replace T cells in the immune response to soluble carrier hapten conjugates by nonantigen-specific mediators that were able to reconstitute the response to SRBC in control cultures (10, 11, 16). This failure has led to the conclusion (10, 17) that for the reconstitution of immune responses to soluble antigens, an antigen-specific T-cell factor is required in addition to the nonantigen-specific one. On the other hand, Amerding and Katz (18) described the reconstitution of the secondary response to DNP-carrier conjugates by allogeneic effect factor (AEF). Recent evidence presented by McDevitt et al. (19), however, suggests that AEF might be a B-cell rather than a T-cell product. Thus, there are at present no unequivocal data demonstrating that a nonantigen-specific T-helper factor, such as TRF, can restore the response to soluble protein antigens in T-deprived cultures.

On the other hand, there is general agreement that as far as the production of TRF-like mediators is concerned, the soluble protein antigen KLH can stimu-
late the generation of such factors in cultures of T cells educated to this antigen
(10, 11, 13). Again, the activity of these factors was demonstrated employing
immune responses to particulate antigens, i.e., heterologous erythrocytes.

In the preceding paper, we have presented evidence suggesting that a nonan-
tigen-specific mediator such as TRF is involved in the anamnestic response to
DNPKLH in vitro (1). In cultures of entire spleen cell populations from mice
primed and boosted with DNPKLH, the DNP-primed spleen cells can be stimu-
lated by DNP on a heterologous carrier to become antibody-producing cells, if
KLH is simultaneously added to stimulate the helper cells. It seemed, therefore,
very likely that a TRF-like mediator is not only produced by antigen-activated T
cells upon rechallenge (10, 11, 13, 14), but that it may indeed mediate a T-cell
helper function in the response to soluble protein antigens.

The question whether the mediator active in this system (1) is identical with
TRF will, eventually, only be answered in a comparative study of a series of
purification steps now available for TRF (Hübner, Müller, Schimpl, and
Wecker, to be published). While these investigations are underway, we have
addressed ourselves to the question if the same activity as defined biologically
is found in both supernates. It is the characteristic feature of TRF that it
reconstitutes the primary immune response to SRBC in spleen cell cultures
devoid of functional T cells best when added 2 days after antigenic stimulation;
addition together with the antigen on day 0 has little effect (3, 7). Table I shows
that supernates derived from DNPKLH-stimulated spleen cell cultures of mice
primed and boosted with DNPKLH meet these criteria; they not only restore the
response to SRBC in nude spleen cell cultures, but also display the same kinetics
of action as TRF. It has been shown previously (7, 20, 21) that this "late" action
of TRF is connected with the maturation of cells that already proliferate upon
antigenic stimulation. The data presented here indicate that such a maturation
signal may also be operative in the immune response to soluble protein anti-
gens.

If so, TRF as routinely obtained by allogeneic (3) or Con A stimulation (4)
might also be able to reconstitute both the primary and the anamnestic response
to DNP-carrier conjugates. This notion was confirmed by the results given in
Figs. 1 and 2, and Table II. As reported by Bluestein and Pierce (22), the
primary immune response to DNPKLH in vitro is rather low, even if entire
spleen cell populations are employed. The response obtained in spleen cell
cultures from nude mice restored by TRF (Fig. 1) is well within that range, even
if FCS is omitted from the cultures for the first 2 days. These responses are
significant, albeit rather low. Much more striking effects can be observed if TRF
is employed to reconstitute the anti-DNP response in T-cell-depleted cultures
from mice primed and boosted with DNPKLH (Fig. 2). The high efficiency of T-
cell depletion in this system by anti-Thy 1.2 plus complement treatment is
reflected by the complete lack of stimulation upon incubation of the cells with
DNPKLH. Background values without antigen, however, tend to be somewhat
higher than those in control cultures, presumably due to B-cell enrichment and
removal of suppressor T cells. Addition of DNPKLH and TRF to these cultures
resulted in an IgG anti-DNP response almost as high as in T-cell-containing
control cultures (Fig. 2). The TRF preparation employed in this experiment had
been chromatographed on a Sephadex G-75 column (6), and the fractions used had been selected for optimal activity in the reconstitution of a primary immune response to SRBC in nude spleen cell cultures. Thus, the biologically active material in both systems may either be identical or at least have very similar molecular weights.

One of the main criteria that a nonantigen-specific helper factor has to meet is the loss of carrier specificity in the anti-hapten response. This important postulate is fulfilled by TRF (Table II). The same TRF preparation as described in Fig. 2 was equally effective in replacing the helper cells whether the DNP-specific B cells were stimulated by the homologous conjugate DNPKLH or by DNPBGG, a heterologous conjugate. Similar results have been obtained by us with TRF derived from allogeneic spleen cell cultures (data not shown) and by North et al. (23).

It has been repeatedly pointed out that TRF does not provide a proliferation signal to B cells but rather gives a maturation signal to already antigen-triggered and proliferating "intermediate" B lymphocytes (20, 21, 24). Without it, the antibody production and secretion does not commence. As shown with SRBC in a primary in vitro system, particulate antigens can trigger a B cell into proliferation and thereby render it responsive to the maturation signal in the absence of any functional T cells (20). Our results with the primary immune response to DNPKLH of nude spleen cultures restored by TRF suggest that the same may hold true to some extent for the soluble T-dependent antigen DNPKLH. In the present and in previous studies on anamnestic IgG responses, spleen cells from primed (15) or primed and recently boosted mice were employed (1, 2). Again, the data are in agreement with the role of TRF as a differentiation signal acting on an "intermediate" B cell (21), in this case induced in vivo. How this intermediate responsive state necessary for the in vitro IgG response is reached, is yet unclear. At any rate, the data presented in this communication demonstrate that in the immune response to soluble carrier-hapten conjugates, TRF, a nonantigen-specific T-cell product, mediates the maturation signal required for the conversion of pre-PFC into both IgM and IgG-antibody-producing cells. These findings suggest that the models elaborated for particulate antigens are also valid for the immune response to soluble protein antigens.

Summary

Spleen cells of dinitrophenyl keyhole limpet hemocyanin (DNPKLH) primed and boosted mice produce a nonantigen-specific helper factor upon in vitro challenge with DNPKLH. This helper factor displays all of the biological characteristics so far described for TRF produced by allogeneic or Concanavalin A stimulation of mouse spleen cells. It restores the primary anti-SRBC response in nude spleen cultures following the same kinetics of action as T-cell-replacing factor (TRF). Conversely, TRF restores the primary in vitro immune response of nude spleen cultures to DNPKLH. TRF also restores the secondary anti-hapten IgG response of T-cell-deprived spleen cell cultures derived from DNPKLH primed and boosted mice. Here the need for carrier specificity is fully overcome. The data therefore suggest that TRF, as a nonantigen-specific maturation factor, mediates the maturation signal required for the conversion of pre-PFC into both IgM and IgG-antibody-producing cells.
signal, is involved in the primary and secondary immune responses to both particulate and soluble antigens.

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