

FEEDBACK INDUCTION OF SUPPRESSOR T-CELL ACTIVITY*

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T-cell-mediated or T-cell-dependent immunosuppression occurs in a wide variety of immunological situations. Those which are antigen-dependent can be subdivided according to the specificity of their effect. Specific T-cell suppression has been shown to play an important role in numerous forms of immunological tolerance (reviewed in 1). In addition, several recent reports have shown that specific suppressor T cells are present in immunized nontolerant animals (2-4). For example, Tada and his colleagues have shown that adoptive transfer of carrier-primed T cells suppressed the antihapten response of normal mice immunized with a hapten on the specific carrier (2). This demonstration is particularly intriguing because the mode of immunization Tada used to produce the specific suppressor T cells was one which should have been optimal for producing helper T cells. This apparent paradox reminded us of the interesting findings of Celada (5), recently confirmed by Bell and Shand (6), which demonstrated the difficulty of adoptively transferring immunity to normal adult mice with cells capable of conferring high levels of adoptive immunity to newborn or irradiated mice. In light of the recent discoveries of cell interactions in the generation of suppressor T cells (reviewed in 1), Celada's results could be explained by the induction of suppressor cells in the normal recipients of the immune cells. A number of studies from our laboratory have also suggested that immune cells can interact with normal cells to produce an antergistic effect; that is, the response of the two cell populations mixed together is less than either one responding alone (7-10). If such antergistic reactions occurred in the mice studied by Tada and his colleagues the apparent paradox would be resolved.

To examine this possibility, we immunized mice by the same techniques used by Tada and Takemori (2) and adoptively transferred the cells to normal and irradiated mice. We confirmed the finding that transfer of carrier immune T cells to normal mice suppresses the response to a hapten on that carrier. In addition, we showed that transfer to irradiated mice, of the same portion of cells that suppresses the response of normal mice, leads to a marked augmentation of the response. The augmentation seen in the irradiated mouse can be suppressed by the addition to that mouse of normal T cells. These results suggest that some cells which have been thought to be suppressor T cells are not, but rather serve as inducers of suppressor T cells in the cell populations with which the test cells are interacting.

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Materials and Methods

Animals. 6- to 8-wk old male CAF₁ mice (BALB/c × A) were obtained from Jackson Laboratories, Bar Harbor, Maine.

Antigen. Keyhole limpet hemocyanin (KLH) was purchased from Pacific Biomarine Co., Venice, Calif. KLH was coupled with 2,4-dinitrophenyl (DNP) hapten by the method of Eisen et al. (11) and DNP₈KLH (eight DNP groups per 100,000 mol wt of KLH) was used in these experiments. *Bordetella pertussis* vaccine was obtained from Eli Lilly and Co., Indianapolis, Ind.

Immunization and Preparation of Cells. Donors of carrier-primed cells were immunized with two intraperitoneal injections of 100 μg of KLH without adjuvant at 2-wk intervals. Animals were killed 2 wk after the second injection. Their spleens were removed, and single cell suspensions were prepared. Normal or carrier-primed spleen cells (5×10^7 cells/0.2 ml) were injected intravenously into normal or irradiated recipients. The irradiated recipients had received 800 R from a Siemens X-Ray machine (Siemens Corp. Iselin, N. J.) 6 h before transfer. All mice were challenged with an intraperitoneal injection of 100 μg DNP-KLH with 10^9 pertussis organisms.

Nylon wool-passed (T-enriched) spleen cells were prepared according to the method of Julius et al. (12). Carrier-primed spleen cells were treated with AKR anti-C3H θ antiserum (anti- θ) and guinea pig complement at 37°C for 1 h (B-enriched spleen cells). This treatment killed approximately 40% of the spleen cells.

Assay for Antibody-Forming Cells. The number of hapten-specific antibody-forming cells was determined on the sixth day after transfer by the hemolytic plaque assay of Cunningham and Szenberg (13). Sheep erythrocytes (SRBC) were coupled with 2,4,6-trinitrobenzenesulfonic acid (Eastman Kodak Co., Rochester, N. Y.), by the method of Rittenberg and Pratt (14). Total plaque-forming cells (PFC) were developed with rabbit antimouse IgG (Miles Laboratories, Kankakee, Ill.). Direct PFC were developed without anti-IgG.

Results and Discussion

When 5×10^7 carrier-immune spleen cells were transferred into normal mice and the recipients immunized with TNP-KLH there was an approximately fivefold decrease in the number of anti-TNP plaques made, compared to animals which received no additional cells (Fig. 1 *a*). The depression was most prominent

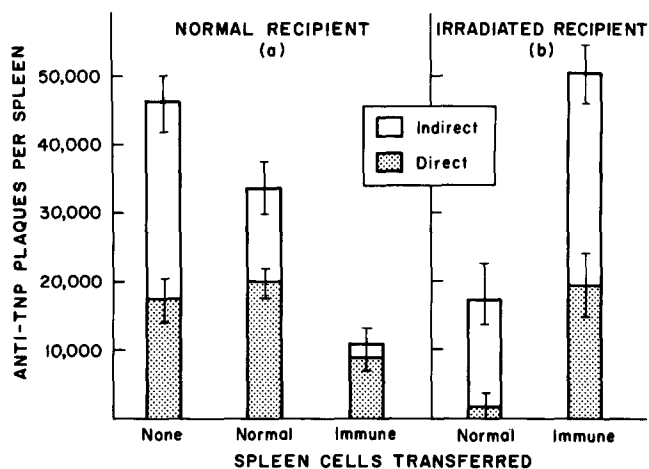


FIG. 1. Effect of transferring 5×10^7 normal or immune spleen cells to normal (*a*) or irradiated (*b*) recipients. A control group (labeled "none") received no spleen cells, and all mice were challenged with 100 μg DNP-KLH. PFC's were determined 6 days after transfer. Dotted area represents direct PFC, and clear area represents indirect PFC. Brackets enclose standard error of the mean.

in the indirect plaques as had been previously noted. There was also the same specificity, ie. there was no depression of the anti-DNP response to DNP-BGG nor were BGG immune cells suppressive for DNP-KLH (data not shown). The adoptive transfer of normal spleen cells produced a slight decrease in the PFC response but this was not statistically significant. When the carrier-immune cells were transferred into irradiated recipients they made a good PFC response which was significantly greater than one made by the normal cells (Fig. 1 b).

Thus, the same population of carrier-immune spleen cells was able to suppress the response of normal recipients and also to make a very good response on their own when transferred into irradiated hosts. This is a clear cut example of an antagonistic interaction between two cell populations; the host was capable of making 5×10^4 plaques to the immunizing antigen, and when repopulated with cells which were also capable of making about 5×10^4 plaques, made 1×10^4 plaques. Thus, the sum of the reaction was less than either of the cell populations responding alone.

To test the cellular basis for this phenomenon, we fractionated the immune spleen cells into a B-enriched population by treatment of the cells with anti- θ serum and complement and into a T-enriched population by passing the spleen cells through nylon wool. The results (Fig. 2) show that adoptive transfer of the unfractionated cells into normal recipients was highly suppressive; that the nylon wool effluent cells were also suppressive, although perhaps not to the same extent as the unfractionated cells; (note: there were more than twice as many T cells

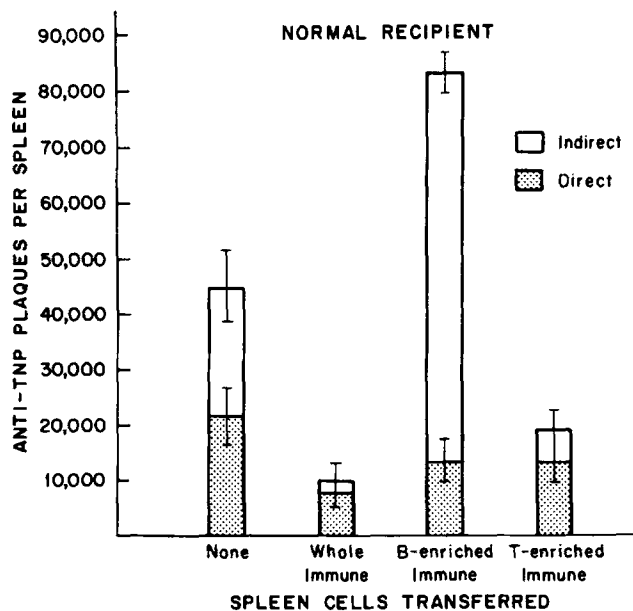


FIG. 2. Effect of transferring 5×10^7 whole or fractionated immune spleen cells to normal recipients. B-enriched cells were from anti- θ -treated spleens and T-enriched cells were from nylon wool-passed spleens. All mice received $100 \mu\text{g}$ DNP-KLH, and PFC's were determined 6 days after transfer. Dotted area represents direct PFC, and clear area represents indirect PFC. Brackets enclose standard error of the mean.

transferred in the nylon effluent), and that T-depleted cells were not only nonsuppressive but were actually augmentative. Whether this augmentation was due to a lowered number of T cells or to the B cells is under investigation at the moment. However, there is precedence for anticarrier antibody augmenting the antihapten response so that it would not be surprising if it were due to the B cells. The important point of this experiment is, however, that the main cell population from the carrier-immune spleen which interacts in an antergistic fashion with the host, is in the T-cell fraction. The reason why the T cells were less suppressive (on a per cell basis) than the whole spleen is not entirely clear. It is possible that there are functional subsets of T cells which stick to nylon wool or it is also possible that T-B interactions between the immune cells affect how they interact with the host cells. We are presently examining these possibilities, but it is clear from the data presented that the immune T cell is the main donor cell partaking in the antergistic reaction.

In another experiment we were able to show that it is also probably the host T cell which participates in the suppressive interaction. Repopulation of irradiated hosts with normal spleen cells, enriched for the T cells by passage through nylon wool, was able to suppress the response of the immune cells from 5×10^4 PFC to 1.4×10^4 (Fig. 3). In other experiments we were able to show that normal B-enriched spleen cells were not suppressive at the same time the T-enriched cells were (unpublished results).

Our results indicate that there are important feedback interactions between T-cell populations. We suggest that when the immune T cell reacts with the carrier it emits signals which are recognized by the normal host T cell causing the induction of suppressor cell activity probably in the latter population. A similar type of activation of suppressor T-cell activity in normal T-cell populations has

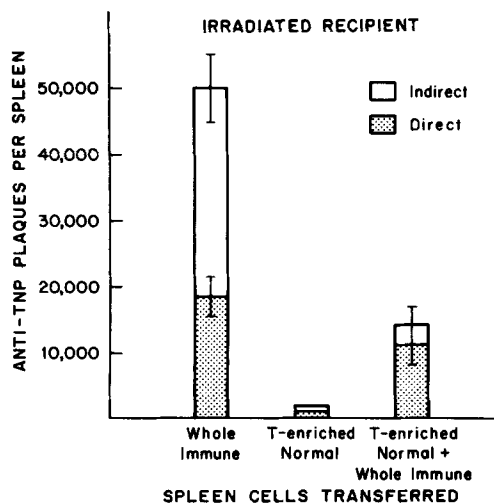


FIG. 3. Effect of transferring 5×10^7 whole immune, 1×10^7 T-enriched spleen cells or a mixture of the two cell populations to irradiated recipients. PFC's were determined 6 days after transfer and brackets enclose standard error of the mean. Dotted area represents direct PFC, and clear area represents indirect PFC.

been shown to be produced by immune B cells (15). These results indicate that the feedback loops which regulate the immune response are quite complex. It is known that the immune response is often a competition between suppression and help (reviewed in 1). There are numerous manipulations which can skew the battle in either direction. In this case, we suggest that too much feedback product is made too early, activating suppressor cells and shutting off the response before it can get started. Our manipulations of the cells probably produced an exaggerated, aberrant form of normal regulation.

Our results also imply that immune T cells are qualitatively different from normal T cells in that they read feedback signals differently. In addition to examples of feedback induction of suppressor T-cell activity in normal but not immune T-cell populations, such as shown in the present study, a similar conclusion can be deduced from studies with passive antibodies. An actively immunized mouse with SRBC antibody titer of 7 (\log_2) will exhibit a good T-cell mitotic response when challenged with an appropriate dose of antigen (16). A normal mouse with a passively acquired antibody titer of 7 will have no mitotic T-cell response to the same dose of antigen (17). Thus, it is clear that immune T cells are better at responding to antigen in the face of large amounts of feedback products than are normal cells.

Lastly, our results serve to emphasize the caution one must exercise when one mixes two cell populations, obtains a suppressive effect and ascribes the suppression to one of the two populations. In some instances, what seems to be a suppressor population acts instead as an inducer of suppressor cells in the second population.

Summary

Adoptively transferred carrier immune T cells interact with nonimmune T cells in recipients in a fashion which generates specific immunosuppression, although both the immune and normal cells function quite well as helper cells when not admixed.

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