Thymus-Derived Lymphocytes: Humoral and Cellular Reactions Distinguished by Hydrocortisone

Abstract. *Lymphocytes derived from the thymus (T cells) take part in the induction of humoral antibody and also effect cell-mediated graft-versus-host reactions. Preliminary treatment of mice with hydrocortisone caused an inhibition of T-cell function in humoral immunity, while enhancing the graft-versus-host reactivity of the same population of spleen cells. This suggests that different types of T cells participate in cellular and humoral immune reactions.*

Lymphocytes derived from the thymus (T cells) play a central role in both cellular and humoral immune responses. The T cells apparently act directly in such cellular reactions as delayed hypersensitivity (*1*), contact-dependent cytotoxicity to target cells (*2*), and graft-versus-host reactions (*3*). In contrast, humoral immunity is mediated by circulating antibodies secreted by lymphoid cells that are not derived from the thymus (B cells). Nevertheless, the induction of specific antibody by B cells against many antigenic determinants requires the cooperation of T cells (*4*). The mechanism by which T cells and B cells interact during induction of the antibody response is not clear. However, it is known that B cells may not produce antibody to haptenic determinants on an immunogen unless other determinants on the immunogenic molecule are recognized by T cells. The part of the immunogen that interacts with T cells is called the carrier. Thus, induction of antibody

filter well technique for the induction of an antibody response to DNP in vitro (*5, 11, 12*). Filter wells contained slices of spleens with 0.01 ml of medium that contained 50 μg of DNP conjugated to RSA. Forty-eight hours later the culture medium was replaced with antigen-free medium. This medium was

synthesis in B cells, against certain haptenic determinants, requires the interaction of T cells with the carrier portion of the immunogen (*5*). In addition, the interaction between the T cell and the carrier, needed for the induction of antibody to hapten, does not appear to require the production of antibody to the carrier antigens themselves (*6*). Hence T cells serve as independent helpers in humoral immunity, as well as agents of cellular immunity. We have designed experiments to determine whether the T cells which mediate cellular immunity are the same T cells which act as helpers in humoral immunity.

The cells which induce graft-versus-host reactions, in mice, are resistant to prior treatment of the animal with hydrocortisone (*7*, *8*). Our experimental approach, therefore, was to give mice prior treatment with hydrocortisone and to measure the ability of their cells to perform both graft-versus-host reactions and humoral immunity functions, which are dependent on T cells. We found that T cells which perform a helper function in antibody responses are separable from T cells that are involved in a cell-mediated graft-versus-host reaction.

We measured T-cell helper function by inducing a primary antibody response in tissue culture against a hapten conjugated to a carrier (*5*). Formation of antibodies to hapten by B cells in this system in vitro depends upon successful prior sensitization of T cells in vivo against the specific carrier. Therefore, induction of antibodies to hapten serves as a sensitive measure of specific T-cell helper function. We used the dinitrophenyl (DNP) group as hapten, and rabbit serum albumin (RSA) as carrier (*9*). Ten male mice of strain C57BL/6j received a 0.1-ml intraperitoneal injection of 2.5 mg of hydrocortisone acetate. Ten control mice were injected with buffer alone. Two days later the mice were injected intraperitoneally with 200 μg of RSA in complete Freund's adjuvant (Difco). The spleens of the animals were removed after an additional period of 2 days. Part of each spleen was assayed for graft-versus-host reactivity (*10*) and part was used in the Millipore

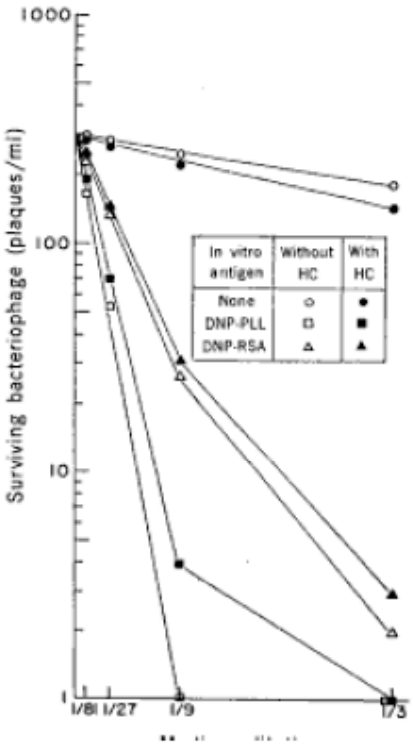
function was inhibited. The average ratio of weights from right and left nodes was 3.2 : 1 in the group of mice which received parental spleen cells that were treated with hydrocortisone. The average ratio was 1.9 : 1 ($P < .05$) in the group of mice that received parental spleen cells that were not treated. An assay with spleen weights to measure the graft-versus-host reaction also indicated that preliminary treatment with hydrocortisone increased the relative graft-versus-host activity of spleen cells (*8*).

Our results support the following conclusions:

1) The graft-versus-host reaction, as observed by others (*7*, *8*), depends upon a population of T cells which are resistant to hydrocortisone.

2) The B cells which synthesize antibodies to hapten are resistant to hydrocortisone. This was demonstrated by the production of antibodies to DNP in response to DNP-PLL which was unaffected by hydrocortisone treatment.

3) The T cells that interact with carrier antigens to serve the helper function are susceptible to hydrocortisone.



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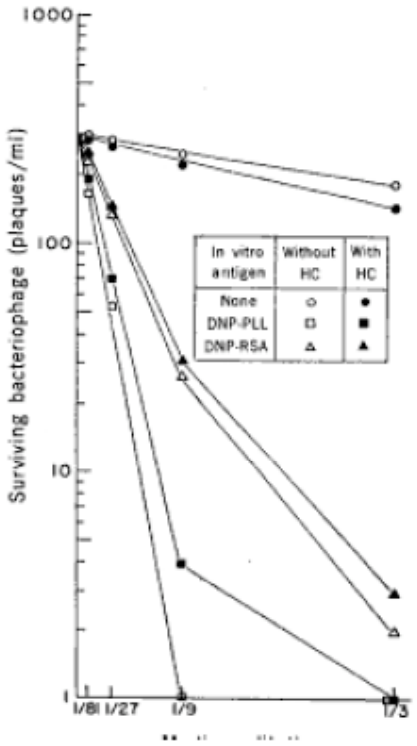

This was shown by failure of spleen cells, first treated with hydrocortisone, to recognize RSA as a carrier for the DNP hapten.

4) The T cells involved in helper function acquire resistance to hydrocortisone after they have been sensitized to carrier antigens. We found that injecting RSA before treatment with hydrocortisone preserved the carrier effect. This may explain why the suppression of antibody formation by adrenal steroids is much less effective when these drugs are administered after antigen stimulation (*15*).

The different susceptibilities to hydrocortisone provide evidence that both the graft-versus-host and helper functions that are dependent on the thymus are controlled by different T-cell mechanisms. It is possible that the thymus can program the differentiation of precursor lymphoid cells into two completely separate populations—a population resistant to hydrocortisone that mediates graft-versus-host and possibly other cell-mediated reactions, and a population sensitive to hydrocortisone that provides the helper function in humoral immunity. On the other hand, as suggested by Raff and Cantor (*16*), different immunologic activities may be performed by T cells as they pass through stages of differentiation. Thus, T cells at an early stage of differentiation might be active as helper cells and mediate graft-versus-host reactions only after further maturation. A third possibility is that the same T-cell type has both functions, but that the mechanisms involved in carrier interaction alone are susceptible to hydrocortisone. This possibility appears less likely because the carrier effect itself is resistant to hydrocortisone once the T cell is sensitized (Fig. 2). Moreover, hydrocortisone appears to increase the graft-versus-host potential of lymphocyte populations by destroying susceptible lymphocytes rather than by merely altering cell functions (*8*). It is unlikely that specific tolerance to RSA could have been induced by injecting RSA in complete Freund's adjuvant.

We have studied the effects of hydrocortisone on the development of lymphocytes which mediate contact-depen-

that are needed to produce cytolysis of target cells may also differ from T cells active in the graft-versus-host reaction. In addition to different susceptibilities to hydrocortisone, T cells active in graft-versus-host reactions and T cells active in cytolysis differ in their response to antibodies against a T-cell antigen *(18)*. Our conclusions pertain to peripheral T cells. Studies of the antibody response of mice to sheep red blood cells suggest that lymphocytes in the thymus may produce a carrier effect that is resistant to corticosteroids *(19)*.

Therefore, at least two, and possibly three, different T cells may be distinguished. Identification of these cell types is important to our understanding of the basic processes of lymphoid cell differentiation and cooperation which are factors both in antibody production and in cell-mediated immune responses. Competition between humoral antibodies and immune lymphocytes may lead to enhanced tumor growth in man *(20)* or to allograft tolerance *(21)*. Therefore, identification and separation of lymphoid cell types ultimately may provide a way of manipulating and controlling immune reactions in patients with tumors or transplanted organs.

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