PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 706–709 (1975)

Steric Hindrance of CON A Receptor Sites by Antigen: a Possible Explanation of Ir Regulated Responses[1] (38614)

MARVIN L. TYAN

Dental Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

In many species the immune responses to a variety of antigens including synthetic polypeptides, weak native antigens and strong native proteins given in limited doses are under the control of autosomal dominant genes (Ir genes) that are linked to the major histocompatibility locus and are expressed in thymus-passaged lymphocytes (T cells) (reviewed in 1, 2). For example, mice from H-2^k strains have better *in vivo* and *in vitro* antibody responses to low concentrations of bovine-gamma-globulin (BGG) or DNP-BGG than do mice from H-2^b strains (3–5), and crosses between them respond to these antigens as well as do the H-2^k parents.

It has been proposed that the products of Ir genes function on T cells as receptors for antigen, that these products are not immunoglobulin in nature, and that the defect in low responder strains is an impairment of the ability to recognize or bind the carrier portion of the antigen (reviewed in 6). However, other studies have shown that antigen binding by T cells can be blocked with anti-immunoglobulin sera (reviewed in 2), that thymocytes from low responder strains possess the capacity to recognize the proper antigen and to react to it to some degree (2, 4), and that H-2 and Ir specificities are not involved in the early phases of antigen binding or recognition (5). On the basis of these observations, it has been proposed (4) that the deficiency in antibody synthesis manifested by low responder strains is the result of an antigen-induced membrane change in T cells that impairs the interaction between T cells and the antibody producers (B cells) with the result that B cell proliferation and antibody production are limited.

This hypothesis has been given some support by the demonstration of a putative antigen recognition unit (B_2-microglobulin, B_2m) that on the basis of tissue distribution alone would appear to be distinct from Ir gene products (7, 8). However, β_2m does appear to have a functional and/or physical linkage with histocompatibility antigens and the receptor site for a T cell mitogen on the membranes of human lymphocytes. It was shown that HL-A and β_2m migrate together in the plasma membrane, and that masking B_2m with specific antisera severely impairs responses to thymus-dependent antigens and to a T cell mitogen, PHA. Related studies have revealed competition between the T cell mitogen Concanavalin A (Con A) and antisera to the H-2 complex (9) or to Ir specificities (Tyan, M. L., manuscript in preparation) for the same or for closely positioned receptor sites on mouse lymphocytes. Taken together, these observations tend to place histocompatibility antigens, Ir gene products, β_2m and the receptor sites for certain T cell mitogens in close proximity on the membranes of T cells. It follows from this that if the ConA binding site represents a receptor complex for a physiological molecule regulating T cell proliferation (11, 12), antigen binding to the putative recognition molecule (β_2m) could conceivably obscure the mitogen receptor to an extent determined by the conformation of the antigen and the genetically-encoded (?Ir-gene) spatial relationship between the receptor site and the recognition unit. If this construction has validity it would be predicted that when spleen cells are stimulated *in vitro* with physiological concentrations of Con A and increasing amounts of thymus-dependent antigens are added to the cultures, the response to Con A, if depressed, would be most severely impaired by the antigen to which cells of that genotype respond most poorly. Presented below are the results of experiments with spleen cells from four

[1] This work was supported by USPHS Research Grant Nos. CA 13216, HD 07708 and DE 02668 from the National Institutes of Health and by General Research Support Grant No. RR 5333.

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strains of mice demonstrating that *in vitro* responses to Con A can be inhibited significantly by relatively low concentrations of two thymus-dependent antigens, and that in the case of C57BL/6 mice this impairment is most marked when the antigen used is one to which this strain responds poorly (DNP-BGG).

Materials and Methods. The donors of spleen cells were adult male CBA/J, C3H/HeJ, B6D2F$_1$ and C57BL/6 mice. C57BL/6 mice respond poorly to BGG and DNP–BGG and well to keyhole limpet hemocyanin (KLH) and DNP–KLH; the other strains respond well to both.

DNP–BGG and DNP–KLH were obtained from Calbiochem, La Jolla, CA, and they were added to the cultures at concentrations ranging between 0.6–100 μg/ml. Con A was purchased from Miles Laboratories, Kankakee, IL. and was used at concentrations of 0.5, 1.0, 2.5 and 5.0 μg/ml. Preliminary experiments indicated that Con A and the two antigens used in these studies do not react directly with one another to any significant degree.

The spleen cells were obtained, dissociated, and suspended in culture medium at a concentration of 2 × 10^6 nucleated cells/ml as reported previously (4). Con A was added to the cell suspensions, and after mixing, 2 ml aliquots were put in 12 × 75 mm polystyrene culture tubes (Falcon Plastics, Oxnard, CA). At this point the antigens were added to the individual tubes. The medium was Eagle's S-MEM, augmented with nonessential amino acids, L-glutamine, 10% heat inactivated fetal calf serum and penicillin and streptomycin (100 units and 100 μg/ml, respectively). The cells were cultured at 37° in 5% CO_2 in air for 3 days without refeeding. The proliferative responses of the cells were measured by ^{3}H-thymidine incorporation into DNA during a 16-hr period (2 μCi/tube; sp act, 6.7 Ci/mmole). The results represent the mean values of three replicate cultures and are expressed as:

1. Cpm/10^3, cells with Con A and antigen.
2. Cpm, cells with Con A and antigen/cpm, Con A only × 100.

All of the experiments to be reported have been performed at least three times, and the results have been in close agreement. Therefore, only representative data will be presented.

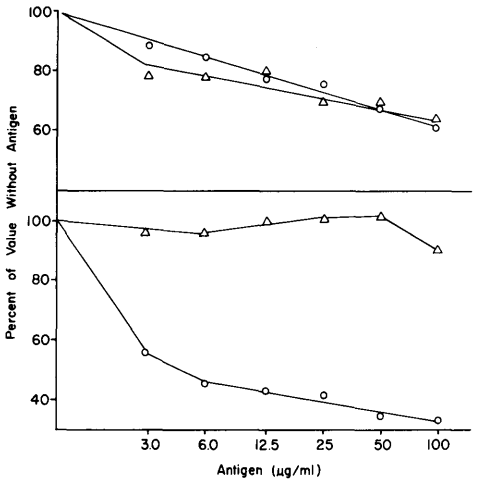

FIG. 1. The effects of increasing concentrations of DNP–KLH (△) and DNP–BGG (○) on the proliferative responses of CBA (upper) and C57BL/6 (lower) spleen cells to Con A stimulation (1.0 μg/ml). The results represent the incorporation of ^{3}H-thymidine in cultures with antigen and Con A/incorporation in cultures with Con A only ×100.

Results. CBA spleen cells cultured with Con A (0.5–5.0 μg/ml) incorporated three to nine times more ^{3}H-thymidine into DNA than did unstimulated cells. The addition of increasing amounts of DNP–BGG or DNP–KLH to these cultures resulted in increasing and parallel depression of the responses to Con A (Fig. 1); overall, DNP–KLH inhibited Con A stimulation slightly more than did DNP–BGG, although frequently the dose-response curves were superimposed. In general DNA synthesis was slightly to moderately depressed by both antigens at their lowest concentrations (0.6–1.5 μg/ml), and thereafter little impairment was noted until antigen reached 25–100 μg/ml. As the levels of Con A were increased, more antigen was required to produce the same degree of inhibition, and at the highest concentration (5 μg/ml) little impairment was seen until antigen exceeded 100 μg/ml (30% inhibition). However, in one experiment CBA spleen cells cultured with Con A, 2.5 μg/ml, were inhibited by relatively low concentra-

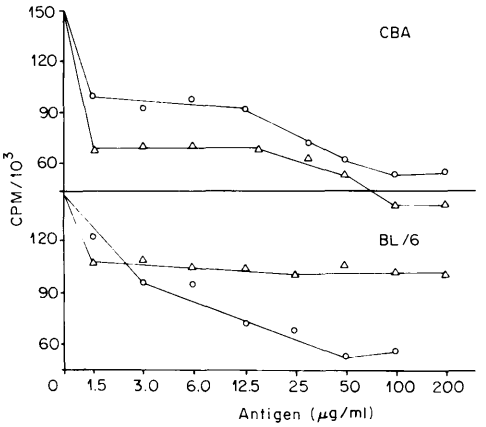

FIG. 2. The effects of increasing concentrations of DNP-KLH (△) and DNP-BGG (○) on the proliferative responses of CBA (upper) and C57BL/6 (lower) spleen cells to Con A stimulation (2.5 μg/ml). The results represent the incorporation of ^{3}H-thymidine (cpm/10^3) by the cells on the third day of culture.

tions of antigen (Fig. 2), but here again DNP-KLH and DNP-BGG produced nearly equal effects. In summary, both DNP-BGG and DNP-KLH interfered with the stimulation of CBA spleen cells by Con A to some degree; however, both antigens impaired Con A stimulation equally and with the exception of one experiment (Fig. 2), the impairment was never severe. The results with C3H/HeJ and B6D2F$_1$ spleen cells were very similar and the data will not be presented here.

In contrast to the results with CBA, C3H/HeJ and B6D2F$_1$ cells, DNP-BGG consistently inhibited C57BL/6 spleen cell responses to Con A while DNP-KLH had almost no effect, (Fig. 1–3). Spleen cells from C57BL/6 mice responded to Con A as well as did cells from the other strains, but as has been reported (3–5), their reaction to DNP-BGG was considerably less than that to DNP-KLH (Fig. 3). The addition of increasing amounts of DNP-KLH to Con A stimulated cultures had almost no effect until the levels of antigen exceeded 50–100 μg/ml; however, as little as 1.5 μg/ml DNP-BGG (Con A, 0.5 μg/ml) inhibited the mitogenic action of Con A on C57BL/6 cells. In general, the degree of inhibition produced by DNP-BGG was inversely proportional to the concentration of Con A,

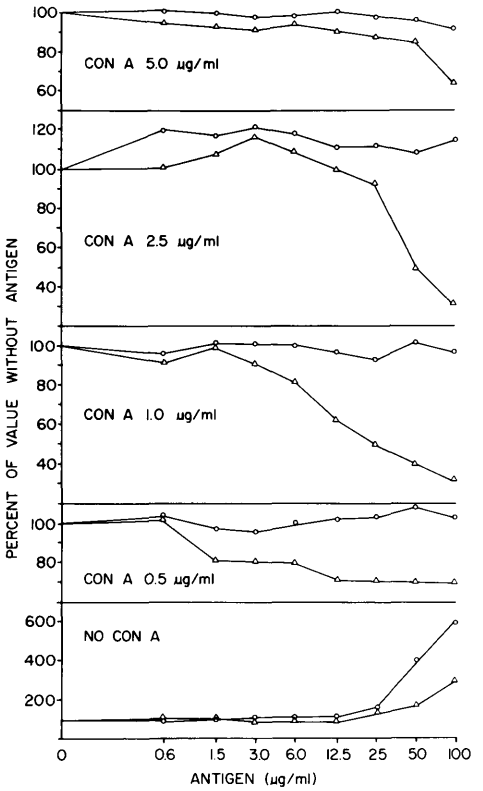

FIG. 3. The effects of increasing concentrations of DNP-KLH (○) and DNP-BGG (△) on the proliferative responses of C57BL/6 spleen cells cultured with antigen alone or with increasing concentrations of Con A (0.5–5.0 μg/ml). The results are based on the incorporation of ^{3}H-thymidine on the third day of culture and are expressed as: cpm with antigen/cpm without × 100 (no Con A) or cpm with antigen and Con A/cpm Con A only × 100.

and as this concentration was increased more antigen was required to produce an equal level of impairment. Maximum inhibition (70%) was recorded with Con A, 1.0 μg/ml; at the lowest level (0.5 μg/ml) maximum depression (30%) was achieved with DNP-BGG, 12.5 μg/ml. Higher levels of antigen presumably induced some cell division.

Discussion. It has been shown that the addition of thymus-dependent antigens to cultures of spleen cells can impair the response to the T cell mitogen Con A to an extent dependent upon the concentration of Con A and antigen and the genotype of the responding cells. CBA, C3H and B6D2F$_1$ mice have comparable antibody responses

to DNP–BGG and DNP–KLH *in vivo*, and the addition of these antigens to cultures of their Con A stimulated spleen cells produced equal impairment of the reaction to Con A. On the other hand, C57BL/6 mice react well to DNP–KLH and poorly to DNP–BGG, and the addition of DNP–BGG to Con A stimulated C57BL/6 spleen cells resulted in a marked impairment of cell proliferation while DNP–KLH had almost no effect. Thus, it has been demonstrated with cells from four strains of mice that two thymus-dependent antigens can interfere with the expression of the mitogenic properties of Con A, and that in one instance an antigen under Ir gene regulation affects cells from low responder strains more severely than those from high responders. The somewhat surprising degree to which both antigens depressed Con A stimulation of spleen cells from all strains tested may be explained by polyclonal T cell binding of the multiple carrier determinants possessed by these large complex molecules, minimal direct reactivity between the mitogen and the antigens, and by T cell feedback inhibition by factors produced in response to Con A/antigen stimulation (13); these complex interrelationships will become amenable to analysis when a simple, chemically-defined antigen under Ir gene control becomes available.

In view of the evidence placing histocompatibility antigens, Ir gene products, a putative antigen recognition unit (β_2m) and the receptor site for Con A in close proximity in the membrane of T cells (7–10), it seems not illogical to assume that they may all be involved directly or indirectly in the regulation of T cell-mediated immune responses. Thus, Ir and H-gene products may in some way determine the spatial relationship between the antigen receptor site on T cells and the Con A binding complex. In certain genotypes, the configuration determined by these genes could allow steric hindrance to occur between an antigen of a particular conformation and the adjacent receptor for a physiological T cell mitogen; presumably, this would result in impaired T cell proliferation and T cell-B cell cooperation. However, this is all highy speculative and while the results reported here are consistent with this hypothesis, these observations must be confirmed by studies with other antigens under Ir gene control and with other strains of mice.

Summary. Spleen cells from mice that respond poorly (C57BL/6) or well (CBA, C3H/HeJ and B6D2F$_1$) to DNP-BGG, an antigen under Ir gene regulation, were cultured with the T cell mitogen Con A and varying concentrations of DNP–BGG and DNP–KLH. It was found that DNP–BGG depressed the responses of C57BL/6 spleen cells to Con A stimulation to a much greater degree than did DNP–KLH; the Con A stimulated responses of spleen cells from the other strains were impaired equally and less severely by both antigens. The possible implications of these findings with regard to Ir gene regulation of thymus-dependent immune responses were discussed.

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Received June 27, 1974. P.S.E.B.M. 1975, Vol. 148.