Brief Definitive Report

GENETIC CONTROL OF LYMPHOCYTE ACTIVATION: LACK OF RESPONSE TO LOW DOSES OF CONCANAVALIN A IN LIPOPOLYSACCHARIDE-NONRESPONDER MICE*

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C3H/HeJ mice do not respond to the polyclonal B-cell activator lipopolysaccharide (LPS) from *Escherichia coli*; this was first described by Sultzer who observed that mice of this strain did not respond to an intraperitoneal (i.p.) injection of LPS as measured by the accumulation of leukocytes in the peritoneal cavity. Neither were C3H/HeJ mice as susceptible to LPS toxicity (1). It was later reported that LPS-induced mitogenesis (2, 3), adjuvanticity (4), and the appearance of Ia antigens on B lymphocytes as induced by LPS, (5) was also absent in C3H/HeJ mice. However, lymphocytes from these mice respond normally to the polyclonal B-cell activators purified protein derivative of tuberculin (2, 6) and dextran sulfate and have also been reported to respond normally to concanavalin A (Con A) (2). Furthermore, the immune responses to sheep erythrocytes (7) and soluble thymus-dependent antigens (4) are normal in C3H/HeJ mice. Unresponsiveness to LPS in C3H/HeJ mice has been found to be due to a defect in a single gene or a set of linked genes (3, 8) which has been mapped between the major urinary protein locus and the locus coding for polysyndactyly on chromosome 4.[1]

We have reported that injection of LPS into mice of an LPS-responsive strain causes a shift in the Con A dose-response curve of cultured spleen cells, suppressing the low dose response (9). Therefore, we tested the Con A proliferative response in cultures of normal or LPS-activated spleen cells from LPS-responder (C3H/Tif) and LPS-nonresponder (C3H/HeJ) mice. We report here that C3H/HeJ spleen cells respond poorly to low concentrations of Con A (0.05–0.1 μg/ml). Injection of LPS 2 days before culture inhibits the response to low doses of Con A in cultures of C3H/Tif spleen cells but has no inhibitory effect on the dose response profile of C3H/HeJ spleen cells. Furthermore, the low dose Con A response of spleen cells is dependent upon the presence of an Ia-positive cell.[2]

[1] Watson, J., K. Kelly, M. Largen, and B. A. Taylor. 1977. The genetic mapping of a defective LPS response gene in C3H/HeJ mice. Manuscript submitted for publication.

[2] Persson, U., P. Bick, L. Hammarström, E. Möller, and E. Smith. 1977. Different require-

The role of Ia-positive cells in the Con A response of C3H/Tif and C3H/HeJ spleen cells is described.

Materials and Methods

Mice.   C3H/HeJ and C3H/Tif mice were used at an age of 4–16 wk if not otherwise indicated.

Polyclonal B- and T-Cell Activators.   LPS from *E. coli* was prepared by Professor Tord Holme (Department of Bacteriology, Karolinska Institutet, Stockholm, Sweden) and was obtained as a lyophilized powder. Con A was purchased from Pharmacia Fine Chemicals, Ltd., Uppsala, Sweden.

Cells and Culture Conditions.   Mouse spleen cells were removed under sterile conditions and pressed through a stainless steel mesh, resuspended with a pipette, and washed with balanced salt solution. The cells obtained after various treatment, were cultured in microcultures (Microtest II; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). 500,000 cells were cultured in Mishell-Dutton medium (10) in a vol of 0.2 ml. All cultures were incubated at 37°C in plastic boxes, and perfused with a gas mixture of 10% CO_2, 7% O_2, and 83% N_2.

Treatment with Anti-Ia Antiserum.   Spleen cells from C3H/HeJ and C3H/Tif mice were treated with A.TH anti-A.TL antiserum (anti-I^k) and guinea pig complement as described for anti-Thy 1.2 serum treatment (11). The cytotoxic titer of the anti-I^k serum was 1/64 for splenic lymphocytes. Normal ATH serum served as a control.

Assay of DNA Synthesis.   During incubation, ^{3}H-thymidine (1 μCi/culture) (sp act 5 μCi/mmol; Radiochemical Centre, Amersham, England) was added to each culture. 1 day later the cultures were harvested (multiple sample harvester; A/S Skatron, Lierbyen, Norway), washed with distilled water, and collected on glass fiber filters. All filters were dried overnight and transferred to scintillation tubes with toluene-based scintillation fluid and radioactivity measured in a scintillation spectrophotometer (Tri-Carb; Packard Instrument Co., Inc., Downers Grove, Ill.).

Results

Failure of C3H/HeJ Spleen Cells to Respond to Low Doses of Con A.   Normal C3H/Tif or C3H/HeJ spleen cells were cultured with various concentrations of Con A in serum-free medium and uptake of ^{3}H-thymidine was measured after 48 h. It was found that C3H/HeJ spleen cells responded poorly to low doses of Con A (0.05–0.1 μg/ml) while C3H/Tif spleen cells gave a vigorous response. It can be seen that both strains show a peak response at 0.5–1.0 μg/ml (Fig. 1).

Response Kinetics of C3H/HeJ and C3H/Tif Spleen Cells after Con A Activation are Similar.   Our initial findings could be explained by a difference in the response kinetics between C3H/HeJ and C3H/Tif spleen cells since DNA synthesis was measured at only one time interval, 48 h. To test this possibility, spleen cells from each strain were cultured with various concentrations of Con A and ^{3}H-thymidine uptake was measured at 24-h intervals from day 2 to day 5. The results of cultures stimulated with low concentrations (0.1 μg/ml, Fig. 2 A) and optimal concentrations (0.5–1.0 μg/ml, Fig. 2 B) of Con A are shown. Spleen cells of both strains show a peak response on day 2 to the optimal Con A dose. However, C3H/Tif spleen cells responded maximally to 0.1 μg/ml of Con A on day 2 while C3H/HeJ spleen cells did not respond after any time period. Thus, the low dose unresponsiveness of C3H/HeJ spleen cells appears to be a defect of this strain and not a result of differential response kinetics of the strains tested.

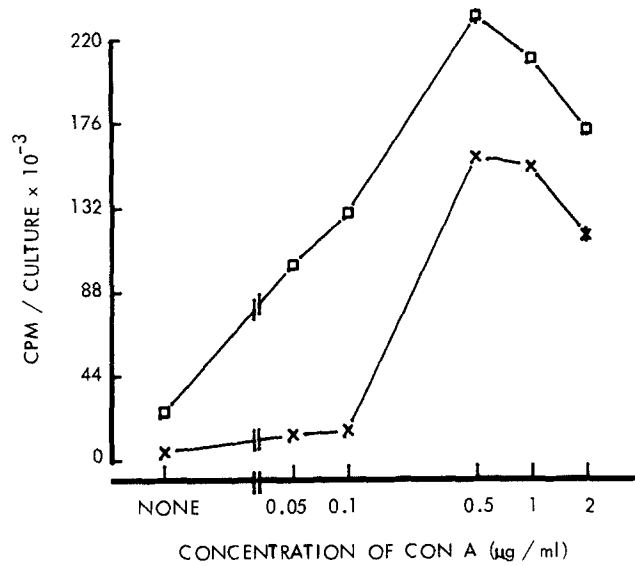*The Proliferative Response Induced by Low Doses of Con A is Suppressed by*

FIG. 1.   Stimulation of C3H/Tif and C3H/HeJ spleen cells by Con A. C3H/Tif (□—□) or C3H/HeJ (×—×) spleen cells were cultured alone or in the presence of various concentrations of Con A. Incorporation of ^{3}H-thymidine was measured after 2 days.
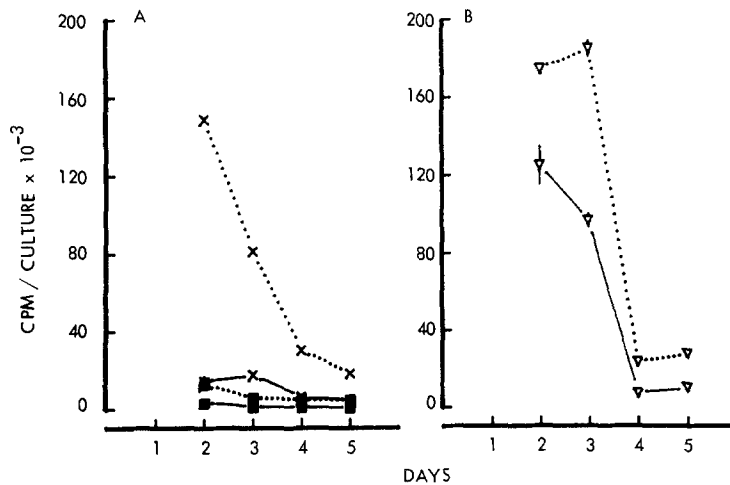

FIG. 2.   Kinetics of Con A activation. C3H/Tif (·····) or C3H/HeJ (—) spleen cells were cultured in the absence (■) or presence (×) of 0.1 μg/ml Con A (A). Spleen cells were also cultured in the presence of optimal Con A concentrations; 0.5 μg/ml (C3H/Tif, ▽·····▽), or 1.0 μg/ml (C3H/HeJ, ▽—▽) (2B). Incorporation of ^{3}H-thymidine was measured on days 2–5.

Prior LPS Activation.   We have reported that spleen cells from mice injected 2 days before culture with 250 μg of LPS responded differently to Con A in vitro than did spleen cells from untreated mice (9). The response to low doses of Con A was inhibited after injection of LPS, whereas the effect on the response to higher doses was variable and often negligible. We have tested the effect of LPS injection on Con A activation in cultures of spleen cells from mice that are either
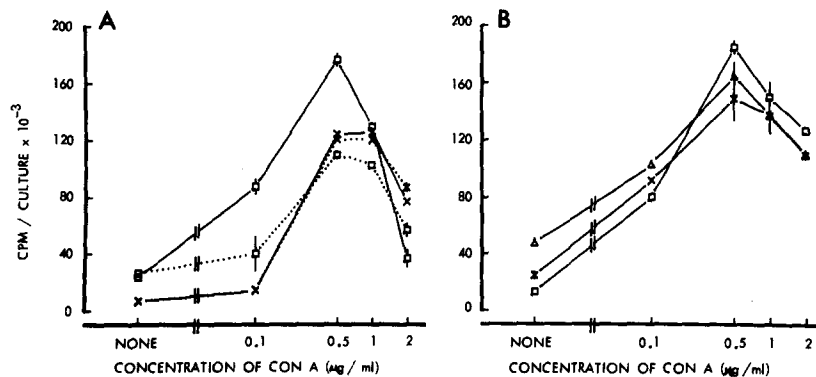
Fig. 3.   Effect of LPS on Con A responsiveness. C3H/Tif (□) or C3H/HeJ (×) spleen cells from normal (—) or LPS injected mice (·····) were cultured with various concentrations of Con A (A). C3H/Tif spleen cells were cultured with various doses of Con A in the absence (□—□) or presence (×—×) of 1 μg/ml, or 10 μg/ml (△—△) LPS (B). Incorporation of ³H-thymidine was measured on day 2.

high responders (C3H/Tif) or low responders (C3H/HeJ) to LPS. As can be seen in Fig. 3 A, normal spleen cells from C3H/Tif mice responded to the low dose of Con A (0.1 μg/ml), whereas spleen cells from mice injected with LPS 2 days before culture did not. As before, spleen cells from LPS low-responder mice did not respond to this dose of Con A, nor did a prior injection of LPS change the dose-response curve to Con A.

It has been reported that T cells respond better to low doses of Con A in the presence of LPS (12, 13). This was now tested with spleen cells from C3H/Tif mice. Normal spleen cells were cultured in the presence of various doses of Con A alone or with 1 or 10 μg of LPS. The results in Fig. 3 B show that with low doses of Con A only additive effects are observed on the proliferative response in the presence of LPS, whereas in cultures with higher doses of Con A, the DNA synthetic responses were less than additive.

Pretreatment of Spleen Cells with Anti-Ia Serum and Complement Causes a Shift in the Con A Dose-Response Curve.   Ia-positive T lymphocytes have been implicated in the response of Ia-negative T cells to Con A (14). Therefore, we tested the effect of removing Ia-positive cells before stimulating C3H/HeJ and C3H/Tif spleen cells with various concentrations of Con A. As shown in Fig. 4, the dose response curve is shifted for both strains of spleen cells. Importantly, however, the response to low doses of Con A (0.05–0.1 μg/ml) was reduced (C3H/Tif) or abolished (C3H/HeJ). The mitogenic response of C3H/Tif spleen cells to LPS was reduced by 50% in this experiment.

Discussion

Spleen cells from C3H/HeJ mice, which are nonresponders to LPS, do not respond to low concentrations of Con A while spleen cells from the *H-2*-compatible LPS-responsive strain C3H/Tif respond well. Spleen cells from C3H/Tif mice that received an injection of LPS 2 days before culture failed to respond to low concentrations (0.05–0.1 μg/ml) of Con A but responded normally to higher concentrations. A similar shift in the Con A dose-response curve is observed in cultures of C3H/Tif spleen cells depleted of Ia-positive cells.
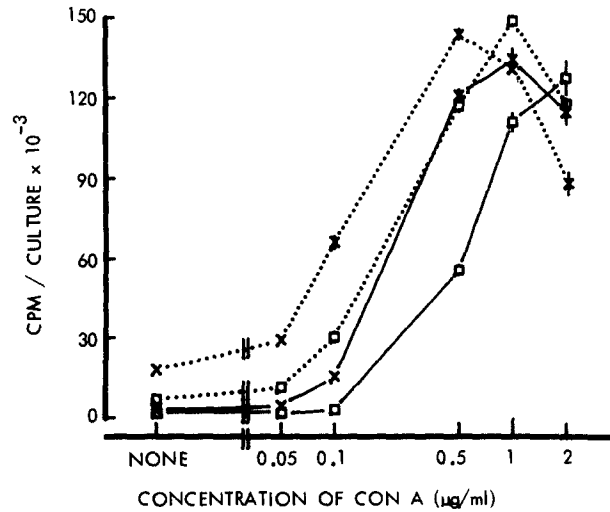
FIG. 4.   Effect of anti-Ia treatment on Con A responsiveness. C3H/Tif (·····) or C3H/HeJ
(—) spleen cells were treated with anti-Ia serum (□) or normal mouse serum (×) and
complement before culture with various concentrations of Con A. Incorporation of ^{3}H-
thymidine was measured on day 2.

These observations, taken together, indicate that either the T cell, responding
to low concentrations of Con A or a required helper cell, is Ia positive, LPS
sensitive, and is absent or not activated in LPS-nonresponder mice. We have
shown earlier that splenic T cells, responding to low concentrations of Con A,
require the presence of serum plus Ia-positive adherent cells contained in the
spleen or peritoneal cavity.

It has been reported that simultaneous activation of T cells by LPS and low
concentrations of Con A leads to an enhanced proliferative response compared to
the response observed after addition of Con A alone (12, 13). LPS, by itself, has
no effect on the proliferation of mouse T cells. Addition of LPS to unseparated
spleen cells in our experiments did not enhance the Con A response as might
have been expected. However, since unseparated spleen cells contain the re-
quired helper cells for the proliferative response to Con A (13),[2] further activa-
tion by LPS might not increase the T-cell response to Con A. Our observation
that injection of LPS before spleen cell culture causes suppression of the low dose
Con A response suggests that some cells involved in the Con A response are
affected in vivo by LPS, leading to a subsequent inability to respond to low Con
A concentrations or to serve as helper cells (9).[2]

In conclusion, T cells responding to various concentrations of Con A have
different activation requirements. This strongly indicates that there are distinct
T-cell subpopulations that are involved in the proliferative response induced by
different doses of Con A. We believe that T cells responding to low concentra-
tions of Con A have an absolute requirement for the presence of an LPS-
sensitive and Ia-positive cell, possibly a B cell. These findings are of particular
interest since both LPS (9) and low concentrations of Con A (15) can induce
suppressor cells which actively inhibit the primary immune response to thymus-
dependent antigens, thus suggesting a similar effector mechanism.

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