Bacterial lipopolysaccharide (LPS) enhances concanavalin A reactivity of thymocytes from the low-LPS-responder mouse strain C3H/Hej[1]

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Summary. The capacity of LPS to enhance Con A reactivity of thymocytes was studied comparatively in the low-LPS-responder C3H/Hej mice and the high-LPS-responder CBA mice. The extent of synergism LPS + Con A was found similar in both strains.

When cultured in the presence of bacterial lipopolysaccharide (LPS), B-lymphocytes from most mouse strains are activated to extensive proliferation[2]. Such a stimulation cannot be detected in the case of mouse thymus cells incubated with LPS alone[2]. However, it has recently been demonstrated that the addition of appropriate amounts of LPS to thymus cells considerably increased their in vitro reactivity to the T-cell mitogen concanavalin A (Con A)[3,4]. The mechanism of this synergistic effect of LPS on Con A-induced DNA synthesis is poorly understood but might involve some interaction of LPS with the thymocyte membrane[4].

C3H/Hej is a unique strain whose B-lymphocytes are refractory to the mitogenic effect of LPS[5–7]. The lack of LPS-receptor structure on the surface of C3H/Hej B-lymphocytes has been invoked to account for their LPS unresponsiveness[5]. The question might thus be raised whether LPS can influence DNA synthetic response of C3H/Hej thymocytes in a manner similar to that found in other mouse strains. This point was investigated in the present work. We have studied comparatively the extents of reactivity to Con A of thymocytes from C3H/Hej and CBA mice in the presence of various doses of LPS.

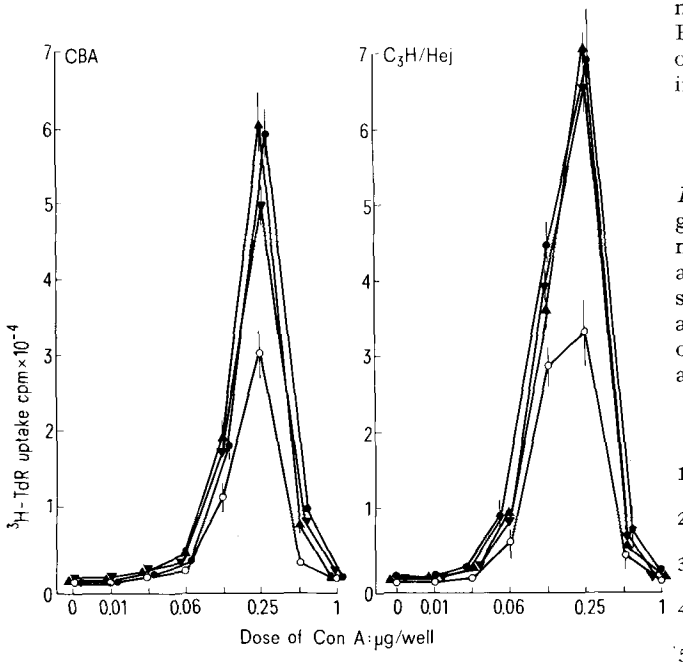

Fig. 1. In vitro responsiveness of CBA and C3H/Hej thymocytes to various doses of Con A added either alone (○) or in the presence of 1.2 (▼), 0.6 (▲) and 0.3 (■) µg of LPS. Each point is the mean ± SE of triplicates.

Material and methods. CBA/Orl and C3H/Hej breeding pairs were obtained from the Centre d'Elevage des Animaux de Laboratoires (CNRS, Orléans-la-Source, France), and the Jackson Laboratories (Bar Harbor, Maine, USA) respectively. Both strains were maintained by strict inbreeding in our animal facilities. All mice were used at the age of 2 months. Thymuses were aseptically removed and dissociated to single cell suspensions. After 2 washings, thymocytes were transferred to RPMI 1640 culture medium (Eurobio, Paris) containing 5% inactivated fetal calf serum. Cultures were set up in Microtest II plates (Falcon 3040) as previously described[8]. Each well received 0.2 ml of cell suspension at a concentration of 2×10^6 cells/ml. Varying doses of Con A (Calbiochem) were added to the cell suspension. LPS from *E. coli* 0.127 B 8 (Sigma), which was verified not to stimulate C3H/Hej spleen cells, was further added in amounts ranging from 0.15 to 10 µg/well.

For purpose of comparison, thymocytes from CBA and C3H/Hej mice were always cultured in the same microtest plate. All cultures were prepared in triplicates.

After 48 h of incubation at 37 °C in a humidified atmosphere of 95% air/5% CO_2, 1 µCi ^{3}H-thymidine (^{3}H-TdR, 25 Ci/mM, CEA, Saclay) was added to each well. After a further 24-h-period of incubation, cultures were harvested using a Multiple Automated Sample Harvester (MASH). Incorporated radioactivity was measured in a Scintillation Counter (Intertechnique, France), and the data expressed as mean CPM ± SE of triplicates. The effect of LPS was expressed as percent increase of Con A stimulation:

$$\frac{(\text{CPM obtained with Con A + LPS}) \times 100}{\text{CPM obtained with Con A alone}} - 100\,.$$

Results and discussion. When cultured for 72 h with graded doses of Con A, thymocytes from CBA or C3H/Hej mice exhibited elevated DNA synthesis which followed a characteristic dose-response profile (figure 1). In both strains, peak response regularly occurred with Con A added in a dose of 0.25 µg/well. By contrast, the addition of LPS alone to thymocytes was unable significantly to augment their ^{3}H-TdR uptake above background level.

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However, when LPS was added to thymocytes in the presence of optimal or suboptimal dilutions of Con-A, this resulted in markedly higher rates of ^{3}H-TdR incorporation than with Con A alone (figure 1). These observations are in agreement with those reported by Forbes et al.[3] and Ozato et al.[4]. Moreover, as shown in figure 2, the degree of enhancement of thymocyte reactivity to Con A depended on the amount of LPS used. Both with CBA and C3H/Hej thymocytes, doses of LPS ranging between 0.15 and 1.2 μg/well were most efficient in this respect, while higher doses (10 μg/well) somewhat exerted inhibitory effects. The above data suggest thymocytes from CBA and C3H/Hej mice are equally sensitive to LPS.

A series of 17 experiments was then carried out to further compare the extents of synergism LPS + Con A in the 2 strains. The results of these experiments are summarized in figure 3. It can be seen that, on average, LPS prove quite as effective in enhancing Con A response in thymocytes from CBA mice as in those from C3H/Hej mice. Thus, the mean increases in Con A response produced by LPS were found to be respectively 98 ± 15% in CBA thymocytes and 94 ± 16% in C3H/Hej thymocytes. Therefore, although C3H/Hej mice are inert to many

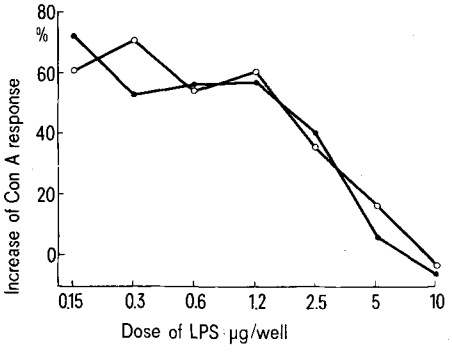

Fig. 2. Synergistic effect of various doses of LPS on the activation by Con A (0.25 μg) of thymocytes from CBA (●) and C3H/Hej mice (○).

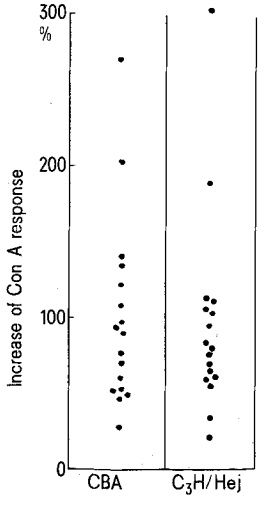

Fig. 3. Maximal degrees of enhancement of Con A response induced by LPS on thymocytes from CBA and C3H/Hej mice. In all 17 experiments, thymocytes from both strains were cultured in parallel under similar conditions.

biological effects of LPS[9], their thymocytes are influenced by LPS in the same way and to the same extent as those from a high-LPS responder strain.

The mechanism of this LPS-induced augmentation of Con A response has not been elucidated. However, the present data definitively rule out the possibility of some participation of contaminating LPS-reactive B-cells in this reaction[3], as it still occurs in a strain devoid of LPS-sensitive B-cells. Rather, it seems that LPS acts on those thymocytes which normally respond to Con A. The mobilization and cross-linkage of glycoproteic Con A receptors at the cell surface accompanied by an increase of membrane fluidity are known to take place early during stimulation by Con A[9]. Such membrane events presumably represent the signal of lymphocyte triggering and LPS might exert its synergistic action primarily at this level. Thus, an interaction of LPS with either the Con A itself or with the thymocyte membrane could be considered.

In the first alternative it is conceivable that the sugar moieties of LPS could bind Con A molecules which in turn would lead to a more efficient cross-linkage of the thymocyte Con A receptors. However, in unpublished experiments, we have found that preparations of LPS that are rendered nonmitogenic by alkaline hydrolysis[11] or treatment with Polymyxin B[12], no longer show synergy with Con A. This requirement for mitogenicity of the LPS argues against a trivial extra-cross-linking of Con A molecules as the mechanism of the synergy. Therefore, the second possibility of some interaction of Lipid-A or of the LPS micelles with the thymocyte membrane would appear more likely. In fact, LPS are amphipathic molecules with a strong tendency to associate with cell membranes[13]. Such molecules are likely to interact in a nonspecific manner with the thymocyte membrane by insertion in the lipid bilayer. The finding that LPS can diminish the surface-charge of cortisone-resistant thymocytes supports this view[14].

Thus, the interaction with LPS might perturb some components of the thymocyte membrane in such a way that the triggering events are enhanced. C3H/Hej mice are believed to be lacking a genetically determined membrane receptor specific for LPS and required for B-cell activation[5]. If one assumes the effect of LPS on thymocytes is mediated through some interaction with their plasma membrane, then the present results implicate that the LPS receptor on B-cells and the LPS receptor on thymocytes are coded by different genes and may be of different nature. Moreover, the synergistic effect of LPS on Con A response can be concluded to proceed by a mechanism different of that involved in B-cell stimulation.

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