

ACQUISITION OF MITOGENIC RESPONSIVENESS BY
NONRESPONDING LYMPHOCYTES UPON INSERTION
OF APPROPRIATE MEMBRANE COMPONENTS*

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Although the detailed mechanism of lymphocyte activation by polyclonal mitogens is far from understood, it is well established that the binding of the mitogen to the cell surface is essential for stimulation to occur (1, 2). Binding by itself is not sufficient to cause cell stimulation, as some lectins (e.g., from *Helix pomatia*) bind to lymphocytes and do not stimulate them (3), whereas others (e.g., concanavalin A [Con A] and the lectin from *Phaseolus vulgaris* and *Lens culinaris*) bind equally well to both T and B lymphocytes, but stimulate only T cells (1, 2). In addition, lipopolysaccharide (LPS), a B cell mitogen, binds to the same extent to B lymphocytes of C3H/eb or C3H/HeJ mice, but the latter cells are not activated (4), although they respond perfectly well to other B cell mitogens such as dextran-sulphate and a purified protein derivative of tuberculin (PPD) (5, 6).

To elucidate the role of the lymphocyte plasma membrane in regulating the response to mitogens, we have used a new technique developed by us for the transfer of membrane components between lymphocytes (7). In this technique, vesicles composed of donor plasma membranes and Sendai virus envelope glycoproteins fuse effectively with recipient cells, resulting in insertion of the donor membrane components into the membranes of the acceptor cells. The ability to transfer important immunological surface markers such as Thy-1, H-2, and receptors for sheep erythrocytes (7-9), as well as to endow the recipient cells with new properties such as the ability to present antigens, has been shown (9). Here we demonstrate that upon insertion of membrane components from lymphocytes responding to mitogens into the membranes of nonresponding cells, the latter could be stimulated by these mitogens. These findings indicate that the inability of either T or B cells to respond to specific mitogens is due to the lack of suitable membrane constituents and that by changing the membrane composition, the lymphocytes can be endowed with new functions.

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Materials and Methods

Cells. Spleen cells were obtained from C3H/eb, C3H/DiSn, or C3H/HeJ mice, 8 wk old, supplied by the Animal Breeding Center of The Weizmann Institute of Science. The splenocytes, depleted from erythrocytes by lysis with 0.83% NH₄Cl, were separated into B and T cells by differential agglutination with soybean agglutinin (SBA) (10). The B cell population was depleted from residual T cells by treatment with anti-Thy-1 antiserum and complement according to Reif and Aller (11). Less than 5% of cross-contamination was found upon analysis on the fluorescence-activated cell sorter of the purified B and T cells, which had been stained with fluoresceinated anti-mouse Ig and anti-Thy-1 antisera.

Preparation of Fusogenic Membrane Vesicles. Purified plasma membranes were isolated from SBA-fractionated T and B splenocytes by the method of Monneron and d'Alayer (12) as described previously (7), where data on the purity of the membrane preparations were also given. In particular, no traces of RNA or DNA contaminants were found. Fusogenic membrane vesicles were obtained by coreconstitution of isolated plasma membranes and Sendai virus envelopes (7). Thus, solubilized plasma membranes (1 mg protein, obtained from 10¹⁰ cells, in 0.3 ml of 1% Triton X-100 in a buffer consisting of 0.1 M NaCl, 50 mM Tris-HCl pH 7.4, and 0.1 mM phenylmethylsulfonyl fluoride) were mixed with solubilized Sendai virus envelopes (1 mg protein, 0.7 ml of 2% Triton X-100 in the same buffer). The mixture was dialyzed in Spectrapore membrane 2 (Spectrum Medical Industries, Los Angeles, CA) for 96 h at 4°C against a large excess of buffer consisting of 10 mM Tris-HCl pH 7.4, 2 mM CaCl₂, 2 mM MgSO₄, 0.02% NaN₃, and 0.1 mM phenylmethylsulfonyl fluoride, in the presence of 1.5 g wet Bio-beads SM-2 (Bio-Rad Laboratories, Richmond, CA) for efficient removal of the detergent. The dialyzed solution was centrifuged at 100,000 g for 3 h at 4°C to obtain co-reconstituted viral plasma membrane (VPM) vesicles. Such vesicles were shown to contain membrane proteins and viral proteins in a ratio of 1:1 (7). Viral vesicles were obtained by the same procedure without the addition of plasma membranes.

Fusion of Vesicles with Lymphocytes. Lymphocytes (10⁷ cells) were incubated with 1.5–5 µg protein (determined as described [13]) of VPM vesicles in 1 ml of fusion solution (0.14 M NaCl, 20 mM Tris-HCl, pH 7.4, 3 mM KCl, 0.8 mM MgSO₄) for 60 min at 4°C with occasional shaking. During this time, the vesicles bound to the cells through the Sendai virus glycoproteins. The cells were collected by centrifugation, resuspended in 1 ml of fusion solution containing 5 mM Ca²⁺, and incubated at 37°C for 30 min to allow fusion to occur. The cells were then washed twice with RPMI 1640 medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY).

Mitogenic Stimulation of Lymphocytes. Untreated or fused lymphocytes were cultured in microtiter plates (3040; Falcon Labware, Oxnard, CA), containing 1 × 10⁵ cells/well in a total volume of 0.2 ml modified RPMI medium (consisting of RPMI 1640 medium supplemented with 5% fetal calf serum [Gibco Laboratories], 5% glutamine [2 mM], mercaptoethanol [5 × 10⁻⁵ M], Hepes buffer [0.5 M], penicillin [100 U/ml], streptomycin [100 µg/ml], and gentamycin [100 µg/ml]). The mitogens were added in aliquots of 10 µl/culture, to a final concentration of 2 µg/ml Con A (Miles-Yeda, Rehovoth, Israel) or 10 µg/ml LPS (*Escherichia coli* 055:B5, Difco Laboratories, Detroit, MI). Cultures were incubated at 37°C with a mixture of 5% CO₂ in air, in a humidified incubator. After 48 h, cells were pulse labeled with 1 µCi/well of [³H]-thymidine (36 Ci/mmol, Nuclear Research Center, Negev, Israel) for 16 additional h. Four replicate cultures were set up in each experimental group. Results are presented as the mean cpm ± SD of each group.

Production of Interleukin-2 (IL-2) Activity. Untreated or fused lymphocytes (10⁷ cells/ml) were cultured in modified RPMI medium with or without 2 µg/ml of Con A and incubated in 60-mm Diam petri dishes (Falcon Labware) at 37°C in a 5% CO₂-air atmosphere. The supernatant was harvested 24 h later, methyl- α -D-mannoside (Pfanstiehl, Waukegan, IL) (20 mg/ml) was added to the supernatant to inhibit Con A activity, and the mixture was filtered through 0.45-µm filters (14). The product designated as "conditioned medium" was stored at -20°C until used. LPS-derived conditioned medium was prepared as Con A-derived conditioned medium, except that the mitogen LPS (10 µg/ml) was removed after 6 h and no methyl- α -D-mannoside was added. Conditioned media from lymphocytes cultured in the absence of mitogen were used as controls.

Assays of IL-2 Activity. IL-2 activity in the conditioned media was examined by the ability

to maintain the growth of Con A-induced T cell blasts (14), prepared by culturing spleen cells (10^7 cells/ml) with 2 $\mu\text{g}/\text{ml}$ of this lectin for 48 h. The blast cells were collected, washed twice with 20 mg/ml methyl- α -D-mannoside and twice with modified RPMI medium, cultured in microtiter plates (10^4 cells/0.1 ml modified RPMI medium), and 0.1 ml of undiluted conditioned medium was added to each well. Culturing was for 48 h, at 37°C in 5% CO_2 -air atmosphere. [^3H]Thymidine was added (1 $\mu\text{Ci}/\text{well}$) 16 h before harvesting. Normal spleen cells mixed with the conditioned media and cultures without addition of conditioned media were used as controls. Four replicate cultures were set up in each experimental group. Results are presented as the mean cpm \pm SD of each group.

Results

The effect of insertion of new membrane constituents into recipient lymphocytes on the ability of the cells to respond to mitogens was examined in three systems. The results presented are of one typical experiment out of four; the range obtained for the four experiments is given in parentheses.

Induction of LPS Responsiveness in Modified C3H/HeJ B Cells. B splenocytes derived from LPS-nonresponder mice, strain C3H/HeJ, were fused with 5 μg protein of VPM vesicles containing B membranes from the LPS-responder strain C3H/eb. The modified lymphocytes were stimulated by LPS to proliferate at a level corresponding to 40% (range 25–50%) of the LPS response of C3H/eb B cells under the same experimental conditions (Fig. 1). When C3H/HeJ B splenocytes were fused with 5 μg protein of VPM vesicles containing T membranes from C3H/eb cells, the modified C3H/HeJ B cells did not respond to LPS. Fusion with the equivalent amount of viral proteins (3 μg) had no effect either (Fig. 1).

Induction of New Mitogenic Responsiveness in Modified T and B Lymphocytes. When B cells derived from C3H/HeJ mice were fused with 5 μg protein of VPM vesicles containing membranes of C3H/eb T cells, net thymidine incorporation into the modified cells was 27% (range 27–50%) of the net response of untreated T splenocytes (Fig. 1). B cells fused with 5 μg protein of VPM containing B membranes responded only poorly to Con A, and fusion with viral vesicles alone did not increase the response to Con A over the background level (Fig. 1). When T splenocytes from C3H/eb mice were fused with 5 μg protein of vesicles containing B membrane from C3H/eb cells, the modified T cells responded strongly to LPS (80% of the LPS response of C3H/eb B splenocytes; range 21–80%). Insertion of viral components alone had no effect either (Fig. 1).

Production of IL-2 Activity by Modified Lymphocytes. Conditioned media obtained from T splenocytes, modified by B membranes and stimulated by LPS or Con A, were assayed for IL-2 activity on T cell blasts. The LPS-derived conditioned medium gave stimulation of the blast cells similar to that obtained by Con A-derived medium from unmodified T cells, indicating strong IL-2 activity (Fig. 2). In contrast, untreated T cells or T cells modified by T membranes produced no IL-2 activity upon LPS stimulation (Fig. 2).

B cells, either untreated or modified by T or B membranes, did not produce any IL-2 activity upon stimulation with LPS (Fig. 2). However, when membranes of T cells were inserted into the B cells, the latter cells acquired the ability to produce IL-2 activity when cultured with Con A, although this activity was lower (up to 30%, range 20–40%) than that produced by untreated T cells. Insertion of T or B cell membrane into T cells resulted in a small decrease in the production of IL-2 activity upon stimulation of the cells by Con A (Fig. 2).

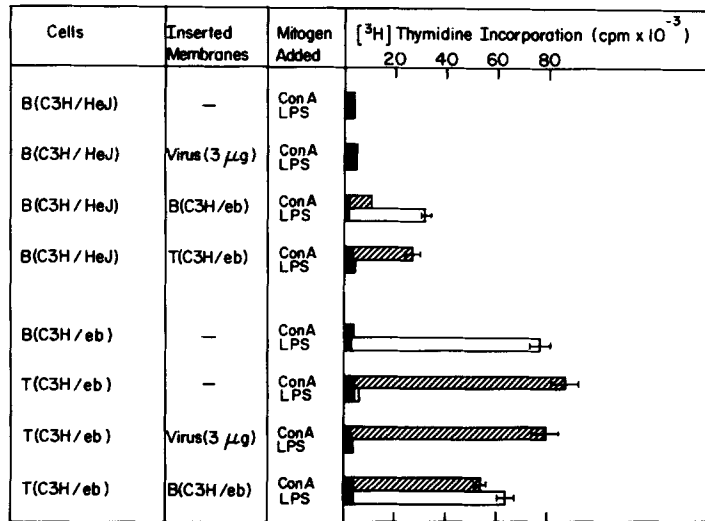


FIG. 1. Induction of mitogenic responsiveness in modified lymphocytes. T or B splenocytes, untreated or fused with viral vesicles (3 μg protein) or with VPM vesicles containing B or T membranes (5 μg protein), were examined for their ability to be mitogenically stimulated by Con A or LPS. Proliferation was measured after 3 d of incubation by uptake of $[^3\text{H}]$ thymidine. Results are given as the mean $\text{cpm} \pm \text{SD}$ of each group. Black bars represent background cpm of cell cultures without any mitogen added. For other experimental details see text.

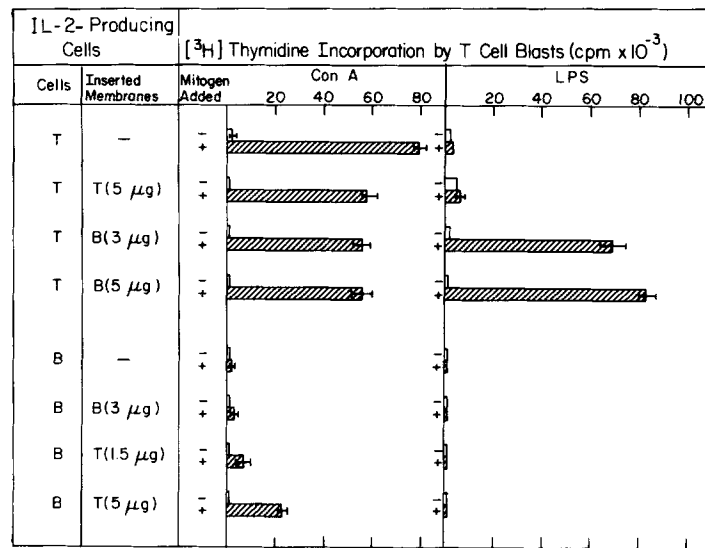


FIG. 2. Production of IL-2 activity by modified lymphocytes. B splenocytes (derived from C3H/eb or C3H/HeJ mice) and T splenocytes (C3H/eb), untreated or fused with different amounts of B or T membranes, were incubated with Con A (2 $\mu\text{g}/\text{ml}$) or LPS (10 $\mu\text{g}/\text{ml}$) under conditions suitable for IL-2 production. Conditioned medium collected after 24 h was assayed for stimulating the proliferation of Con A T blasts. Proliferation was measured after 2 d of incubation by uptake of $[^3\text{H}]$ thymidine. Results are given as the mean $\text{cpm} \pm \text{SD}$ of each group. For other experimental details see text.

Discussion

The findings described above prove that plasma membrane components regulate the response of lymphocytes to mitogenic stimuli. The ability to induce mitogenic responsiveness to LPS in C3H/HeJ B cells by inserting B cell membranes from the responder strain C3H/eb indicates that the recipient cells were defective only at the plasma membrane and that the defects could be repaired by suitable modification of the membrane composition (Fig. 1). Moreover, the response properties of lymphocytes could be altered according to the source of the donor membranes (Figs. 1, 2). Thus T cells, upon fusion with B membrane components, acquired responsiveness to B cell mitogens (LPS) and B cells, when fused with T membranes, could be stimulated by a T cell mitogen (Con A). In all cases the acquisition of mitogenic responsiveness resulted from the insertion of membranes derived from responding cells, whereas insertion of "self" membranes of nonresponding cells or viral components alone did not affect the acceptor cell properties. Thus, B cells from both the LPS-responder C3H/eb or the nonresponder C3H/HeJ strains acquired the ability to respond to Con A upon their fusion with T membranes. Furthermore, the response of T cells, fused with B membranes, to LPS was expressed not only in cell proliferation, but also in production of IL-2 activity, similar to the response of T cells to Con A, and both new functions were expressed to the same extent.

It should be noted that B cells modified with T membranes were stimulated by Con A to produce IL-2 activity, whereas in earlier work (14) and as observed by us, B cells stimulated by LPS did not produce similar activity or any other growth factor. These results raise the possibility that in modified B cells, Con A activates metabolic pathways other than those involved in B cell activation by B cell mitogens such as LPS.

Another conclusion from our findings is that the response obtained depends on both the intrinsic properties of the cells and the presence of a triggering unit, which consists of appropriate receptors and the transducer of the mitogenic signal. T cells modified with B membranes produced IL-2 activity and proliferated in response to LPS, whereas B cells modified with B membranes responded to LPS only by proliferation. It appears that the nonresponsiveness to certain mitogens reflects the absence of this triggering unit at the plasma membrane, whereas the activation machinery at the cell interior is functional in nonresponding lymphocytes.

Our observations are in accord with the recent results of Watanabe and Ohara (15). They concluded that the lack of response to LPS by C3H/HeJ splenocytes may be due to a deficiency in the cell membrane or the cytoplasm but not in the nucleus. Our approach directly proves that the inability of C3H/HeJ B cells to respond to LPS is due to membranal defects.

The ability to endow the lymphocytes with new properties and functions by changing their membrane composition opens new possibilities to elucidate the role of specific membrane constituents in cell maturation, differentiation, and commitment to specific pathways. For example, it would be of interest to examine by this approach whether abnormal cells, whose maturation process is arrested, can be directed to proliferation and subsequently to maturation by changing their plasma membrane composition. In addition, the use of isolated membrane components will allow the identification of the specific membrane constituents which function, either as receptors or as transducers, in cell activation.

Summary

The effect of insertion of plasma membrane components from lymphocytes responding to mitogens into the membranes of nonresponding cells using Sendai virus envelopes as vehicles was examined. T cells modified by B membranes were stimulated by lipopolysaccharide (LPS) to proliferate as well as to produce interleukin-2 activity. B cells modified by T membranes were stimulated by concanavalin A to proliferate and to produce interleukin-2 activity. B cells derived from C3H/HeJ LPS-nonresponder strain of mice, when modified by B membranes derived from the LPS-responder C3H/eb strain, acquired LPS responsiveness. These findings indicate that the inability of either T or B cells to respond to specific mitogens is due to the lack of suitable plasma membrane constituents and that by changing the membrane composition the lymphocytes can be endowed with new functions.

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