THE EFFECT OF MEMBRANE STABILIZING AGENTS ON INDUCTION OF THE IMMUNE RESPONSE

I. Effect of Lymphocyte Activation in Mixed Lymphocyte Reactions*

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In vitro studies of the mixed lymphocyte reaction (MLR) and subsequent cell-mediated lympholysis (CML) (1, 2) have been useful in identification and investigation of the cell populations involved in the allograft response (3-5). As both the inductive and effector phases of the in vitro response require recognition of the histoincompatible cells through membrane-associated receptors, workers have further approached the delineation of the these populations on a pharmacological basis, through the use of chlorpromazine, a membrane-stabilizing drug, to inhibit induction and/or effector function (6). Results have shown that chlorpromazine inhibits both MLR and MLR generation of cytotoxic lymphocytes, the latter by blocking a postproliferative membrane-mediated event required in the generation of cytotoxic effector lymphocytes. In contrast, the drug was shown to have no effect on the recognition, binding, or lysis of target cells by already sensitized lymphocytes. It was postulated that the drug affected the T-cell population capable of cell division upon recognition of LD or Lad cell surface structures and by some surface-associated mechanism prevented subsequent development of cytotoxic effector cells capable of recognizing the SD surface components. The present report deals with experiments designed to test other membrane-stabilizing drugs, imipramine and lidocain, to see whether they exert similar effects to those described for chlorpromazine.

Materials and Methods

Drugs. Chlorpromazine, imipramine, and lidocain were purchased from Leo (Helsinborg, Sweden), Ciba-Geigy (Basel, Switzerland), and Astra (Sodertalje, Sweden), respectively, and were maintained in balanced salt solution (BSS) at 0°C in stock solutions before use in the MLR assay.

Cell Preparations. Spleen cell suspensions from either C57BL or CBA mice were obtained by pressing the spleens through stainless steel grids under sterile conditions. The resultant suspensions were pipetted vigorously and cellular aggregates allowed to settle for 5 min. The cells were then washed twice with BSS and resuspended to a final concentration of 10^7/ml in RPMI media containing 5% heat-inactivated fetal calf serum, 100 IU penicillin/ml and 100 μg streptomycin/ml, and 5×10^{-5} M 2-mercaptoethanol. Cells designated as stimulators in the MLR were irradiated with 1,400 R using an Elma X-ray source emmiting 50 R/min.

Mixed Lymphocyte Reaction. Spleen cell suspensions from C57BL mice (10^7/ml) were set up in

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Falcon Microtest 11 tissue culture plates (Falcon Plastics, Oxnard, Calif.) in a 0.05-ml vol. The stimulator cells (10^7/ml CBA-irradiated spleen cells) were added to the responder cells also in a 0.05-ml vol. All groups received 0.02 ml complete RPMI media. The drugs were added to the cultures in a 0.02-ml vol at various time intervals after the initiation of the MLR. Controls consisted of similar mixtures differing only by the inclusion of C57BL splenic lymphocytes as the stimulator cells. The cultures were placed in plastic boxes, gassed with a 10% CO_2, 7% O_2, and 83% N_2 mixture, and incubated at 37°C for 72 h. [^{3}H]thymidine (0.02 ml, 25 μCi/ml) was then added and the cultures incubated for a further 24 h, at which time they were harvested on a Skatron multiple cell-culture harvester (Skatron A-S 3401 Lierbyen, Norway). The samples were counted on a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Warrensville, Ind.). MLR and control groups were assessed for the total counts per minute (cpm)-thymidine uptake as well as viable cells/culture.

Results and Discussion

Effect of Membrane-Stabilizing Agents on the Proliferative Response in the MLR. To compare the effects of imipramine and lidocain to those caused by chloropromazine, one-way MLRs were set up as described in the Materials and Methods section. The concentrations of drugs added to both the control and stimulated cultures were determined previously and represented the doses that caused suppression of mitogenic activation of cells (L. Hammarström, manuscript in preparation). The drugs were added at 0, 24, 48, and 72 h after initiation of the response.

Fig. 1 shows the effects of chlorpromazine on proliferation in MLR after various time periods in culture. Chlorpromazine at 1.6 μg/ml (5×10^{-6} M) only inhibited the MLR significantly when present in the cultures for 96 h. The inhibition in these groups did not correlate with decreased numbers of viable cells. However, the inhibition of the MLR after inclusion of the drug at 16 μg/ml appeared to be caused by toxic properties of the compound. Thus, MLR responses, control background cpm, and presence of viable cells in the cultures were abolished if the drug was present longer than 24 h, marginal responses and viable cell numbers being seen before this time. The findings could be explained in one of two ways. There could be a time requirement for expression of toxicity on the responding cells in culture. This period would be about 24 h, as a 50% decrease in viable cell number is seen at this time. Otherwise, it is possible that cells in advanced stages of activation (72 h) are less susceptible to the toxic effects of the drug. At present we are not able to distinguish between these two possibilities. Fig. 2 and Fig. 3 show comparative studies using the membrane-stabilizing drugs imipramine and lidocain. It can be seen from Fig. 2 that imipramine at 10 μg/ml (3.2×10^{-5} M) inhibits the MLR if present in the culture for at least 48 h. In these groups the inhibition seen could be explained by a decrease in the number of viable cells. Imipramine at concentrations of 100 μg/ml was totally toxic if present in culture for longer than 24 h. Similarly, in Fig. 3, concentrations of lidocain of 1.0 mg/ml (4.3×10^{-3} M) and 10 mg/ml inhibited the MLR, the inhibition seen at the latter concentration again correlating with total absence of viable cells.

The inhibition of the MLR affected by low concentrations of the three drugs was paralleled in two instances by a decrease in the number of viable cells per culture. This decrease could be explained in several ways: either the drugs are directly toxic to the responding cells in culture or the drugs function as was

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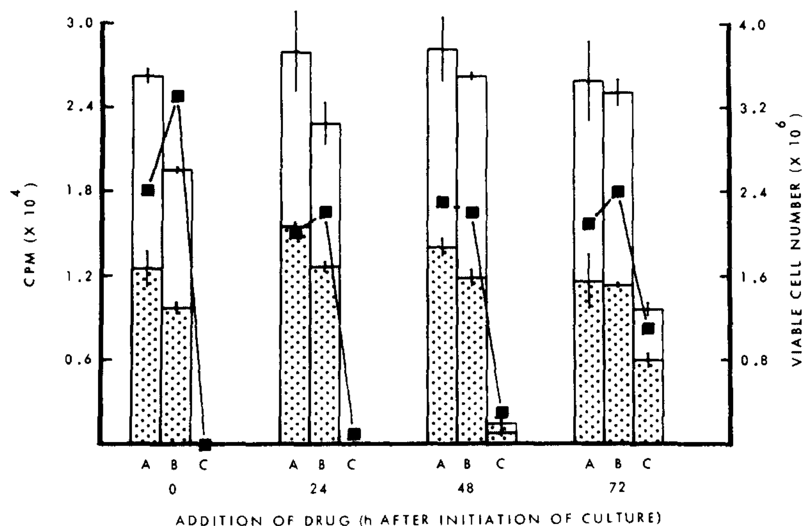

FIG. 1. The effect of chlorpromazine on activation of C57BL splenic lymphocytes in the one-way MLR. C57BL spleen cells were activated in culture by either X-irradiated C57BL or CBA stimulator cells. The drug was added to the MLR 0, 24, 48, or 72 h after initiation of the cultures. The reaction was tritiated after 72 h and harvested 24 h later. At this time all groups were assessed for total thymidine uptake as well as the number of viable cells. The results represent the mean cpm and standard error from both control cultures (C57BL stimulator cells) (⊡) and activated cultures (CBA stimulator cells) (□) with either (A) no chlorpromazine, (B) chlorpromazine at 1.6 μg/ml, or (C) chlorpromazine at 16 μg/ml, the drug being added at the time indicated. The corresponding viable cell number/milliliter in the activated cultures are also shown (■—■).

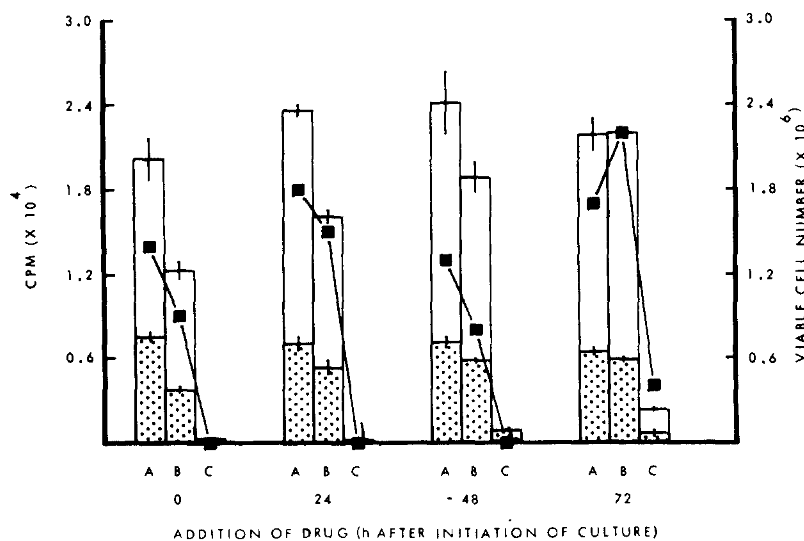

FIG. 2. The effect of imipramine on activation of C57BL splenic lymphocytes in the one-way MLR. The experimental design was set up as described for Fig. 1. The results represent the mean cpm and standard error from both control cultures (C57BL stimulator cells) (⊡) and activated cultures (CBA stimulator cells) (□) with either (A) no imipramine, (B) imipramine at 10 μg/ml, or (C) imipramine at 100 μg/ml, the drug being added at the times indicated. The corresponding viable cell number/ml in the activated cultures are also shown (■—■).

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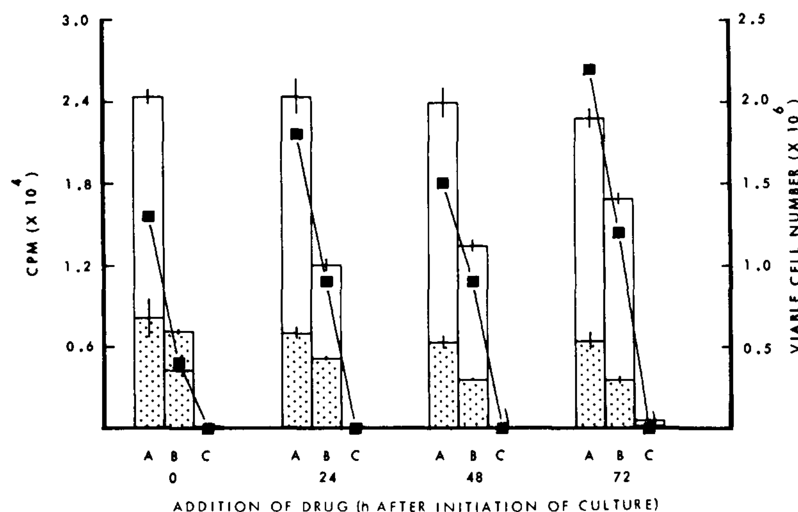

FIG. 3. The effect of lidocain on activation of C57BL splenic lymphocytes in the one-way MLR. The experimental design was set up as described for Fig. 1. The results represent the mean cpm and standard error from both control cultures (C57BL stimulator cells) (▦) and activated cultures (CBA stimulator cells) (□) with either (A) no lidocain, (B) lidocain at 1 mg/ml, or (C) lidocain at 10 mg/ml, the drug being added at the times indicated. The corresponding viable cell number/ml in the activated cultures are also shown (■—■).

previously postulated by blocking cellular activation, thus affecting DNA synthesis and cell division. We feel that the first possibility is only tenable as the decrease in cell number with 10 μg/ml imipramine and 1 mg/ml lidocain occurred also in control cultures (C57BL vs. C57BL irradiated, data not included). However, to further test this possibility the MLR was set up including doubling dilutions of the three drugs at the initiation of the cultures. It was reasoned that if cellular activation was blocked due to a direct toxic effect, a linear decrease in the number of viable cells would be seen with increasingly higher concentrations of the drugs. Conversely, if the drugs blocked activation by a nontoxic mechanism, as has been shown for hydroxyurea, DNA synthesis would be inhibited and viable cell number would remain constant at various concentrations higher than that used for the maximal suppression. As can be seen from Fig. 4, the decrease in cellular activation during the MLR correlated completely with the loss of viable cells from the cultures.

The doses of the various membrane-stabilizing drugs required to attain suppression in the MLR differed considerably. However, in each case, suppression was concomitant with the loss of cells capable of synthesizing DNA at the end of the culture period and can only be explained as being due to the toxicity of the compounds. The differences between these findings and those reported previously, indicating that suppression of the MLR is not caused by a direct toxic action of the drugs, can not be explained at the present time.

We feel that these studies serve to indicate the pitfalls involved in interpretation of data derived from experiments of this type. It is imperative when using such drugs for studying a defined lymphocyte function to control that the

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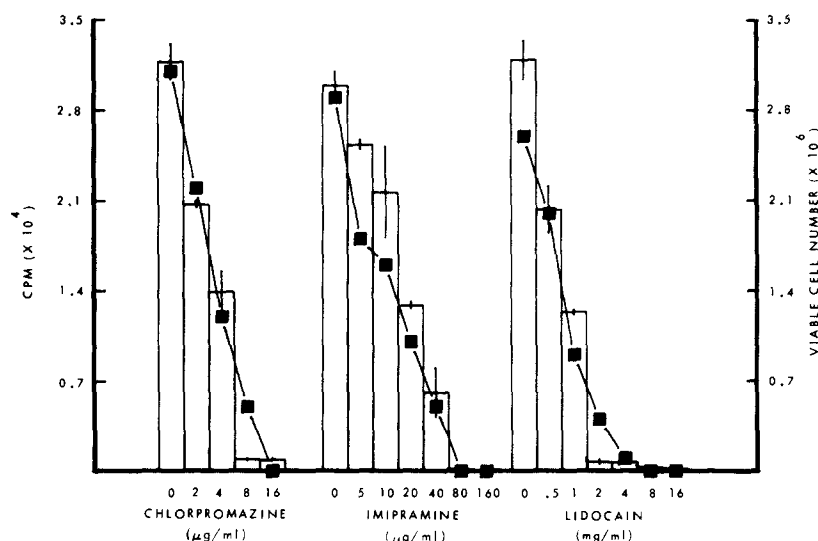

FIG. 4. The effect of chlorpromazine, imipramine, and lidocain on activation of C57BL splenic lymphocytes in the one-way MLR. C57BL spleen cells were activated in culture by X-irradiated CBA stimulator cells. The drugs were added to the MLR at the initiation of the cultures. The results represent the mean cpm and standard error from activated cultures, the drugs being added to the MLR in the concentrations indicated. The mean cpm and standard error from control cultures without the drugs included was 9,898 ± 736. The corresponding viable cell number/ml in the activated cultures are also shown (■—■).

biological effects measured can be explained only by a direct effect on the function investigated and not to more indirect effects induced by the drug.

Summary

The effect of three membrane-stabilizing agents, chlorpromazine, imipramine, and lidocain, on in vitro activation of cellular responses was studied using the one-way MLR. Cellular activation was found to be depressed by these drugs. However, the degree of suppression paralleled the decline of viable cells in the cultures. It is concluded that drug-induced inhibition of MLR was due solely to the toxicity of the compounds.

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