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Levamisole Augmentation of Lymphocyte Hyporesponsiveness to Phytohaemagglutinin in Patients with Pulmonary Tuberculosis (39292)

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It has been reported that levamisole ((tetrahydro-6 phenylimidazo (2, 1-b)thiazole)) augments lymphoid cell mediated immunity *in vivo* (1). The mode of action of the drug has not been clearly demonstrated (2). We have found that an *in vitro* effect of levamisole could be demonstrted using lymphocytes with impaired responsiveness to phytohaemagglutinin (PHA; 3). In a study of lymphocyte function in patients with pulmonary tuberculosis (PTB) we found lymphocyte hyporesponsiveness to PHA stimulation in 65 out of 151 patients (43%). In this paper we report the effect of levamisole on *in vitro* responsiveness to PHA of lymphocytes from PTB patients.

Materials and methods. Thirty-three pulmonary tuberculosis (PTB) patients were studied. None of the patients were receiving chemotherapy at the time of study. There were 18 pretherapy patients with minimal (4), moderate (9), and advanced (5) disease stages and 15 post-therapy patients (3 months to 3 years after cessation of conventional autituberculosis drugs). Lymphocytes were separated by a Ficoll-Isopaque density gradient method (4). Activation of these lymphocytes by phytohaemagglutinin ("purified" PHA, Burroughs Wellcome England), and by pokeweed mitogen (PWM, Gibco, Grand Island, New York) was quantitated by net percentage incorporation of the γ-emitting isotope 75 Seleno-methionine (75Se-Me Amersham, England) as a measure of *de novo* protein synthesis. Briefly, lymphocytes (0.4×10^6) in 1 ml of methionine-free Eagle's medium (Commonwealth Serum Laboratories, Melbourne, Australia) supplemented by 10% fetal calf serum (FCS) and 0.05 μg/ml of unlabeled methionine were cultured in round bottom glass tubes for 72 hr at 37° in a humidified atmosphere of continuously flowing 5% CO_2/95% air. PHA (2.5 μg) and 75Se-Me (0.02-0.03 μCi; 10,000-15,000 cpm) were added at the commencement of culture. In some cultures, PWM (50 μg/ml) was used instead of PHA. At harvest the cultures were centrifuged in an MSE minor centrifuge (2000 rpm/15 min) and the supernatant was separated. The cell pellet was washed twice with 1.5 ml of phosphate-buffered saline (PBS; pH 7.3) and the washings were added to the separated supernatant. The cell pellet and the supernatant were separately treated with 1% perchloric acid (PCA). The sedimented precipitates were washed twice with 1% PCA and counted in a gamma spectrometer (Packard Tricarb Model 3002).

To make comparison between experiments and the responses of different subjects possible, the results were expressed as the percentage incorporation of 75Se-Me instead of in counts per minute. All results were corrected for the amount of PCA-precipitated radioactivity in control tubes containing media alone, and the percentage of 75Se-Me incorporated into the cell and supernatant compartments added to give a total percentage incorporation. The response of lymphocytes to PHA or to PWM was expressed as net percentage 75Se-Me incorporation (stimulated minus control cell cultures).

Lymphocytes (2×10^6) in 1 ml of Eagles' medium-10% FCS were preincubated with levamisole (Jannsen Pharmaceutical, Beese, Belgium) at 37° with concentrations between 0.05 and 500 μg/ml for varying periods up to 24 hr. The cells were washed twice before culture. Untreated lymphocytes were preincubated with media alone.

Results. Preincubation with levamisole had no detectable effect per se on lymphocytes from PTB patients over the dose range tested (Fig. 1). However, the response of

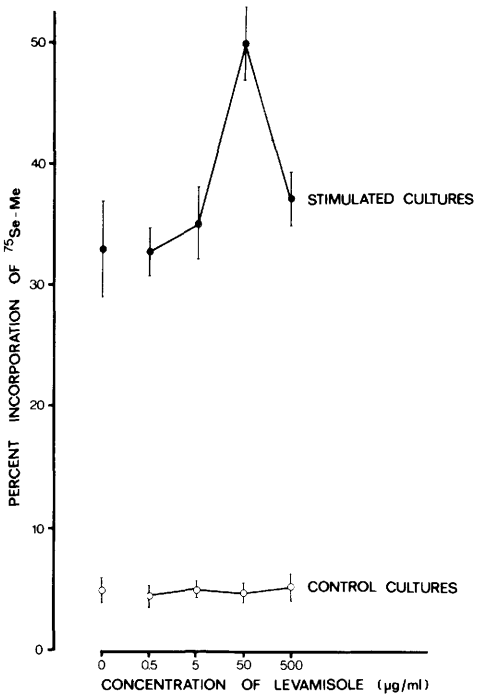

FIG. 1. Effect of 18 hr of preincubation with different concentrations of levamisole on lymphocyte response to PHA. Stimulated cultures contained 2.5 μg/ml of PHA. Each point represents mean ± 1 SD from three or four cultures.

lymphocytes to PHA stimulation was augmented by levamisole at a concentration of 50 μg/ml. Figure 2 shows the effect of varying the preincubtion time with levamisole to subsequent stimulation by PHA and pokeweed mitogen (PWM). The augmentative effect of PWM occurred within 1 hr, whereas the effect of PHA was not apparent before 6 hr, reaching a maximum by 18 hr. In Table I, we show a comparison of the effect of 18 hr of preincubation with that of concurrent addition of levamisole on lymphocyte responsiveness to PHA. An augmentative effect was observed in both situations, but was more pronounced with preincubation.

The effect of levamisole on lymphocytes from 33 PTB patients and 10 normal blood donors were studied using these conditions (preincubation with 50 μg/ml for 18 hr). Using lymphocytes of 42 blood donors, the mean ± 1 SD of net 75 Se-Me incorporation following PHA stimulation was previously found to be 48.3 ± 8.4% (Chan, Chew, and Simons, unpublished data). Hyporesponsiveness to PHA stimulation was defined as a response more than 2 SD below the mean (i.e., <31.5%). In replicate cultures, 1 SD about the mean was 2.5% or less; therefore, a difference of greater than 2 SD (>5%) in lymphocyte response between levamisole-treated and untreated cultures was interpreted as a positive effect by levamisole.

Figure 3 shows the effect of levamisole on lymphocytes from 33 PTB patients and 10 blood donors in relationship to the PHA responsiveness of untreated lymphocytes. Lymphocytes from the 10 blood donors showed responses to PHA within the normal range which were not significantly affected by levamisole. Of the 33 PTB patients, 14 (42%) showed lymphocyte hyporesponsiveness to PHA stimulation. The lymphocyte response of 9 (64%) of the 14 patients was augmented by levamisole. Levamisole treatment restored the lymphocyte

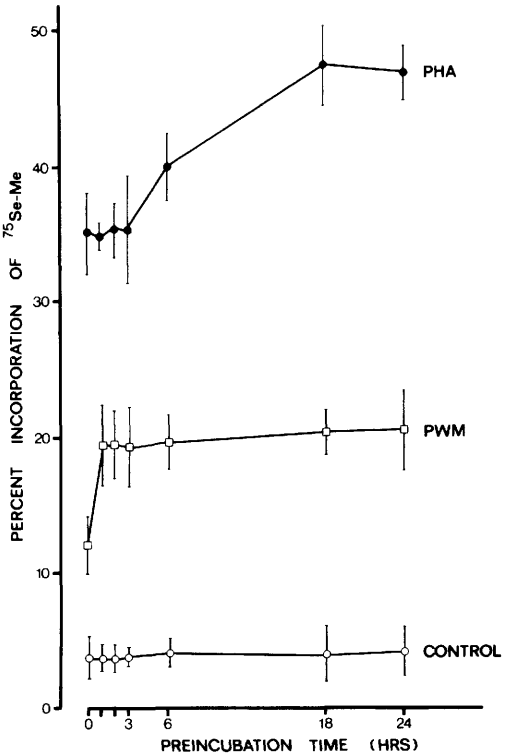

FIG. 2. Effect of varying preincubation time on lymphocyte response to PHA (2.5 μg/ml) and PWM (0.05 μg/ml). Lymphocytes were from A PTB patient and levamisole was used at a concentration of 50 μg/ml.

TABLE I. COMPARISON OF CONCURRENT AND PREINCUBATION TREATMENT WITH LEVAMISOLE ON THE RESPONSE OF LYMPHOCYTES TO PHA STIMULATION.

Treatment	Levamisole (50 μg/ml)	Percentage incorporation of 75Se-Me	
		PHA stimulated (cpm)	Unstimulated (cpm)
Concurrent	–	40.2 ± 2.6 (5025 ± 325)	6.0 ± 1.6 (750 ± 200)
	+	52.6 ± 2.4 (6575 ± 300)	5.6 ± 2.1 (704 ± 262)
Preincubate (18 hr)	–	41.0 ± 2.0 (5125 ± 250)	5.4 ± 1.6 (675 ± 200)
	+	66.1 ± 3.2 (8263 ± 400)	5.6 ± 1.0 (700 ± 125)

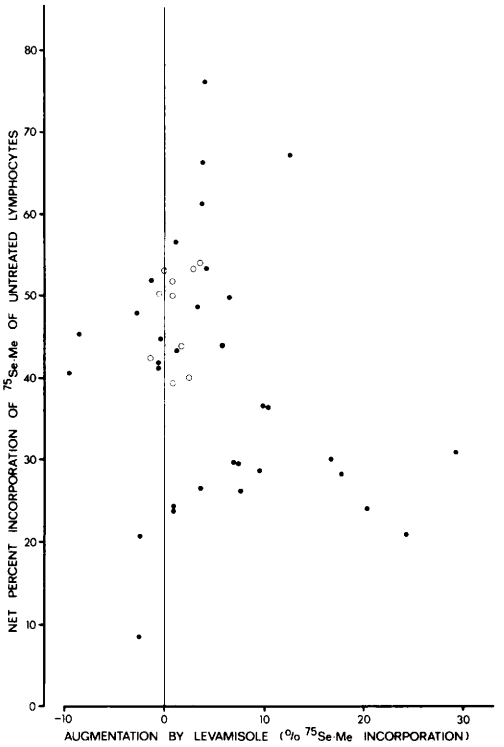

FIG. 3. Effect of levamisole on lymphocyte from PTB patients (●) and normal subjects (○) in relation to PHA responsiveness of untreated lymphocytes.

response to PHA to within the normal range in all 9. Among the remaining 19 patients whose lymphocytes showed a response within the normal range, augmentation associated with levamisole treatment occurred in 5 (26%). In the total of 14 patients whose lymphocytes were augmented by levamisole, the mean ± 1 SD augmentation was 13.4 ± 7.4% incorporation of 75Se-Me (range 6–29.4%). In 2 of the 19 normoresponsive patients, an inhibitory effect was observed.

Discussion. Previous studies on levamisole and lymphocyte function *in vitro* have been interpreted as showing negative or unimpressive effects (2). We have shown an effect of levamisole on *in vitro* lymphocyte responsiveness to PHA stimulation in pulmonary tuberculosis. The augmentative effect of levamisole was most pronounced in lymphocytes showing hyporesponsiveness to PHA. Lymphocytes from 64% of PHA-hyporesponsive PTB patients were augmented by levamisole as compared to 26% of PHA-normoresponsive patients. This difference is statistically significant ($x^2 = 4.76$, $P < 0.05$). However, if hyporesponsiveness is defined by a response more than 1 SD below the mean of normal subject (i.e., <39.9% 75Se-Me incorporation), the difference is more marked ($x^2 = 6.79$, $P < 0.01$). Similar augmentative effects of levamisole on hyporesponsive lymphocytes have been observed in patients with nasopharyngeal carcinoma (Chan, Chew, and Simons, unpublished data).

The present culture system might be suitable as an *in vitro* assay of the lymphocyte augmentative effect of levamisole. It also may be useful in elucidating the mechanism of PHA hyporesponsiveness in that an augmentative effect indicates that PHA-responsive cells are present and that their responsiveness can be partially or completely restored.

Summary. Levamisole has been shown to

augment the *in vitro* responsiveness of lymphocytes to PHA stimulation. The response of lymphocytes of 14 (42%) of 33 patients with pulmonary tuberculosis was augmented by levamisole. Augmentation was observed in 9 (64%) of 14 PHA hyporesponsives lymphocytes and in 5 (26%) of 19 normoresponsive lymphocytes.

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