

The Effects of Autologous Plasma on Human Lymphocyte Transformation (34709)

ROBERT H. ALFORD

(Introduced by Roger M. Des Prez)

Veterans Administration Hospital and Department of Medicine,
Vanderbilt University School of Medicine,
Nashville, Tennessee 37203

Plasma from persons with a variety of disorders has been reported to affect *in vitro* lymphocyte transformation. Plasma samples from individuals with infections (1, 2) ragweed sensitivity (3), multiple sclerosis (4), chronic uremia (5), and cancer have been reported to suppress transformation. Suppression by plasma from tuberculous individuals is reportedly specific; that is, the effect is only on tuberculin-induced transformation (1). Plasma from patients with histoplasmosis has been reported to suppress transformation induced by histoplasmin (2) and plasma from persons with ragweed sensitivity, in a like manner, has suppressed ragweed extract-induced transformation (3). The specificity of these effects is uncertain since multiple antigens were not tested. In addition, lymphocyte cultures without added antigens have been suppressed (4) or stimulated (7) by plasma. Even the potent mitogenic stimulus of phytohemagglutinin (PHA) is diminished by plasma from uremic (5) and cancer (6) patients.

It is likely that suppression of lymphocyte transformation by plasma from persons with various disorders is a result of multiple factors. Therefore, as a background for further investigation, a study of the effects of plasma from normal individuals on lymphocyte transformation was performed. The effects of autologous normal plasma on thymidine uptake of unstimulated, antigen-stimulated, and PHA-stimulated transformation were determined in the present study.

Materials and Methods. Heparinized (20 U/ml) venous blood from three normal subjects was sedimented by centrifugation at 400g for 15 min. RBC were aspirated by

pipette with minimal disturbance of the buffy coat until a packed cell volume of 10–15% remained. The remaining cells were resuspended in plasma and sedimented by gravity for 1 hr at 37°. The leukocyte-rich plasma was separated and sedimented by centrifugation at 250g for 10 min. Leukocytes were resuspended in 3 ml of plasma, applied to a polypropylene column (0.025-ml micropipette, Cook Engineering Co.) containing 400 mg of cotton (8) previously wetted with warm media 199. Following 20-min incubation at 37° the nonadherent lymphocytes were eluted with media 199. The cells were washed 3 × in media 199 and resuspended in the media. The cell suspension contained approximately equal numbers of RBC and leukocytes which were 96–100% lymphocytes. Cultures of 10⁶ lymphocytes in 2 ml of media 199, and the indicated concentrations of autologous plasma were established in screw-capped tubes. Stimulants were PHA-P (Difco, control 528063) or a *Candida albicans* extract (Hollister Stier, lot D252763). The *C. albicans* extract was dialyzed 4 × against 50 vol of saline, once against Hanks' salt solution, Millipore-filtered (0.45-μ poresize), frozen, and finally thawed prior to use. The protein content of the dialyzed *C. albicans* extract (candida) as determined by the Lowry method (9) was 210 μg/ml. Control cultures were incubated a total of 4 days, PHA-stimulated cultures 4 days, and candida-stimulated cultures 6 days. Twenty-four hr before harvest 2.0 μCi of tritiated thymidine (New England Nuclear) was added to each culture. Thymidine uptake was determined essentially as outlined by Newberry *et al.* (2) using volumes of reagents appropri-

ate for smaller cultures added directly to the culture tubes. Uptake was uniformly expressed as counts per minute (cpm) of 0.2 ml of the 0.5 ml of hydroxide of Hyamine (Packard Instrument Co.) digest and therefore represented $\frac{2}{5}$ of the activity of the entire culture. All cultures were run in duplicate and the results were averaged.

The PHA concentration producing maximum lymphocyte transformation was determined for each concentration of plasma by addition of serial dilutions of PHA to a series of cultures. The concentrations of PHA are expressed as milliliters of undiluted reconstituted PHA-P per 2 ml of culture.

Results. The effect of plasma concentration on thymidine uptake of unstimulated lymphocytes is noted in Fig. 1. Maximum uptake

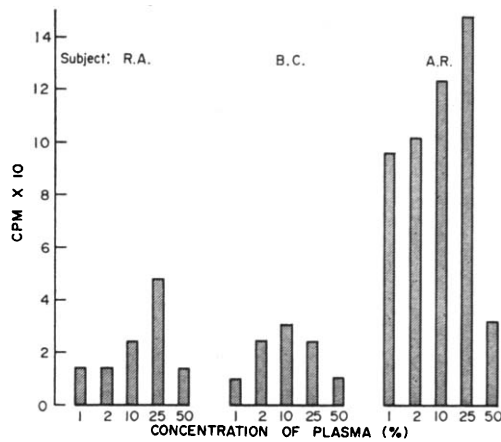


FIG. 1. Effect of autologous plasma concentration on tritiated thymidine uptake of unstimulated human lymphocytes from three normal subjects.

occurred at 25% plasma concentration in two subjects and 10% in the third. Considerable suppression of maximum uptake occurred in a 50% plasma concentration in all subjects.

Increased thymidine uptake over unstimulated controls (3025 vs. 480 cpm) occurred upon PHA stimulation in a system designed to be plasma-free as is indicated in Fig. 2. It is likely that plasma components were adsorbed to the lymphocytes despite 4 washings with 50 vol of media. Considerably increased thymidine uptake occurred in the

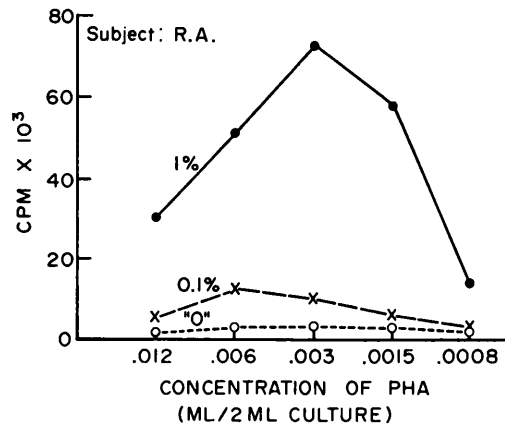


FIG. 2. Thymidine uptake of normal lymphocytes induced by graded concentrations of PHA in the presence of no added plasma, 0.1% plasma, and 1.0% plasma.

presence of 0.1% autologous plasma and optimum stimulation (see below) occurred in the presence of a 1% plasma concentration in the subject illustrated.

PHA-stimulation of lymphocyte thymidine uptake varied markedly with different plasma concentrations as is noted in Fig. 3. For each plasma concentration there was a certain PHA concentration above and below which less than maximum stimulation occurred. The maximum uptake for each plas-

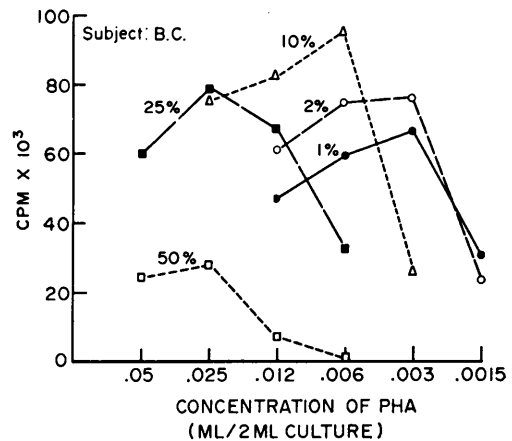


FIG. 3. Effect of plasma concentration on thymidine uptake of lymphocytes stimulated by different concentrations of PHA. The plasma concentration is indicated by the percentage beside each curve. All tests were run concurrently.

ma concentration and the amount of PHA required in each of three subjects is shown in Fig. 4. Optimum stimulation occurred in the presence of 1% plasma in 2 subjects and in 10% plasma in the third. Only $\frac{1}{2}$ to $\frac{1}{8}$ the amount of PHA producing maximum uptake in the presence of 25% plasma was needed for optimum stimulation with the lower plasma concentrations. Increasing plasma concentration above that at which optimum stimulation occurred produced progressive inhibition resulting in decreasing uptake over the entire range of PHA concentrations.

Candida-stimulated thymidine uptake increased with increasing concentrations of the antigen at all plasma concentrations, in contrast to the diminished uptake resulting from high concentrations of PHA. Lymphocytes from two subjects were maximally stimulated by candida in 25% plasma and one in 2% plasma. The plasma concentration allowing optimum PHA stimulation and that producing maximum candida stimulation differed considerably in 2 of the 3 subjects.

Discussion. The results recorded here demonstrate both stimulating and suppressive effects of normal autologous plasma on human lymphocyte transformation. The precise role of plasma in promoting transformation is not known. Studies in tissue culture have

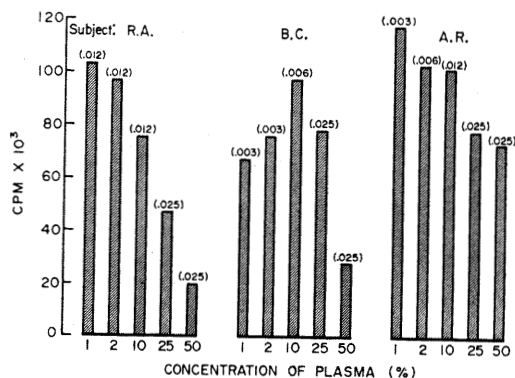


FIG. 4. Effect of plasma concentration on thymidine uptake of lymphocytes from three subjects maximally stimulated by PHA. Numbers in parentheses indicate the amount of PHA required for maximum stimulation at the respective plasma concentrations. Note that the graph, depicting the response of subject B. C., was derived from the information presented in Fig. 3.

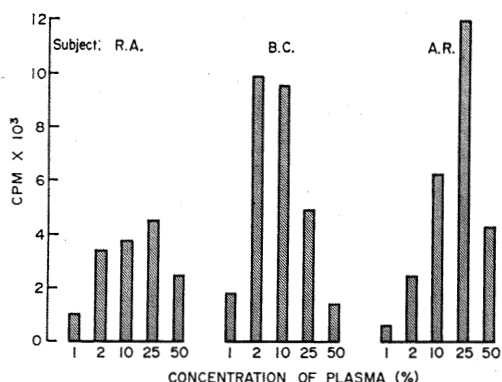


FIG. 5. Effect of plasma concentration of thymidine uptake of lymphocytes stimulated by *C. albicans* antigen (candida).

demonstrated that adhesion of nontrypsinized cells to glass required a serum concentration of approximately 1%. This adhesion-promoting activity was located in the α -globulin fraction (10). The requirement for a similar plasma concentration for optimum PHA-induced thymidine uptake in certain subjects may reasonably be attributed to the same sort of activity promoting adhesion of the mitogen to cells or the cells to each other. Adhesion of cells is known to affect immune responses *in vitro* (11).

Several studies analyzing the effects of varying concentrations of PHA on lymphocyte transformation have demonstrated that higher concentrations of the mitogen are toxic (12, 13). The relative concentrations of PHA and serum in the culture mixture have been reported to affect lymphocyte RNA synthesis *in vitro* (14).

In the present study an optimum PHA concentration was defined for each plasma concentration. In a like manner, when the concentration of PHA was constant, a plasma concentration could be defined above or below which suboptimum stimulation occurred. The inhibitory effects of larger plasma concentrations suggest several possible explanations. Since α -2 globulins have been shown to precipitate PHA (15), plasma protein may combine with PHA so as to interfere with its action on lymphocytes. It is also possible that plasma may inhibit PHA-induced transformation directly because of a normally con-

tained lymphocyte or macrophage reactive factor or indirectly due to formation of a cytotoxic product from the plasma-PHA interaction. The complex PHA-plasma-lymphocyte interactions demonstrated in the present studies do not indicate which or how many of these mechanisms might be operating.

Recently much interest has focused on the role of α -globulins in immunosuppression (16). These proteins have been reported to inhibit both antigen-induced and PHA-induced lymphocyte transformation *in vitro* (17), but were without effect on unstimulated lymphocyte cultures (17, 18). Macrophage immobilization studies have suggested that α -globulin may react in early steps of antigenic recognition (18). Further, it has been proposed that polyribonuclease activity may mediate α -globulin immunosuppression (19).

Suppression of candida-induced transformation by large plasma concentrations in the present study may in part be a result of specific antibody against *C. albicans*. The data presented herein do not prove or refute this notion. However, the reaction tested here was a secondary response, and in most models the primed immune system is relatively resistant to suppression by antibody (20). Also, since unstimulated as well as candida-stimulated cells demonstrated diminished thymidine uptake in plasma concentrations greater than the optimum, it appears likely that at least a part of the suppression was not antibody mediated.

Although the factors involved are incompletely defined, suppression of *in vitro* lymphocyte transformation by normal autologous plasma and the variation in this activity between individuals may be an important phenomenon pertinent to *in vivo* control mechanisms limiting the degree of lymphocyte transformation.

Summary. Autologous plasma was demonstrated both to sustain and inhibit human lymphocyte transformation *in vitro*. A plasma concentration of less than 1% was unable to adequately sustain PHA-induced transforma-

tion. However, by careful adjustments of PHA concentration, a plasma concentration of from 1 to 10% yielded optimum PHA-induced stimulation in the subjects tested. Optimum plasma levels were higher (10–25%) for candida-stimulated and for unstimulated lymphocyte transformation. Suboptimum stimulation resulted when greater concentrations of plasma than the optimum were employed regardless of the stimulant. Considerable variation occurs between normal individuals in autologous plasma suppression of *in vitro* lymphocyte transformation.

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