IN VITRO INTERACTIONS OF MIXED AND UNMIXED SYNGENEIC AND ALLOGENEIC MOUSE MACROPHAGES.

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Three criteria of macrophage activation were measured in mixed and unmixed cultures from inbred and outbred mice. Mixed and unmixed cell cultures displayed no difference with respect to survival in culture, phagocytic rate, or pinocytic rate. Macrophages gave similar results whether cultured in syngeneic or allogeneic combinations. The results suggest that the pooling of macrophages from different animals is an innocuous procedure when the cells are harvested without the use of an exudate-inducing agent. Therefore, experiments with pooled cells should yield data equivalent to that obtained using cells from individual animals.

INTRODUCTION

The pooling of macrophages from several animals is a common procedure in the study of the functions of these cells. It is often necessary because the numbers of cells from a single animal prove too few to serve in extensive or multifaceted experiments. This is particularly true for experiments with alveolar macrophages.

However, this pooling procedure is subject to criticism. First, mixed lymphocyte reactions occur in cultures composed of cells from animals of sufficient genetic dissimilarity (1, 2). Such reactions can result in the formation of lymphokines which, in turn, affect a wide range of macrophage functions, including functional, metabolic, and biochemical changes (3). Since with available techniques macrophage preparations cannot be completely freed of lymphocytes (4), pooled macrophages might behave differently than cells from individual animals. Second, allogeneic macrophages can elicit lymphokine production by lymphocytes (5). Third, in response to allogeneic cells, macrophages can themselves produce cytostatic, and possibly other, effects independent of lymphocyte activity (6, 7).

The use of inbred strains of animals does not necessarily circumvent the above criticisms. Genetic variation in such strains is decreased, but not necessarily eliminated (8). Variation in the genetic constitution may arise by mutation, migration, selection, or human error (9). In addition, the existence of "developmental thresholds" may result in the variable expression of an otherwise equivalent set of genes due to shifts in minor, and largely obscure, environmental factors (10, 11).

The following study was undertaken to determine whether or not mixing macrophages from different animals was sufficient to alter their behavior *in vitro*.

MATERIALS AND METHODS

Normal, uninfected mice of both sexes were used. Swiss outbred mice (Lab Supply Co., Inc.) and an in-house colony of inbred A/J mice were used when six to twelve weeks of age.

Hank's Balanced Salt Solution (GIBCO) containing no calcium or magnesium was used in the cell collection procedures. Cultures were maintained in Medium 199 (M199–GIBCO). All media were supplemented with 10% fetal calf serum, 100 units/m*l* of penicillin, and 50 μg/m*l* of streptomycin (GIBCO).

Alveolar macrophages were collected by the method of Rusell and Roser (12). Peritoneal macrophages were collected, without the use of an inducing agent, by the method of Tolnai (13). After collection, cell suspensions were immediately plated in plastic culture dishes (COSTAR-6, 35 mm). "Individual" cultures consisted of a cell suspension from a single animal. "Mixed" cultures consisted of a mixture of equal volumes of cell suspension from each of two animals. After three hours, non-adherent cells were removed by two to three washings with phosphate-buffered saline, pH 7.1. Before and after washing, incubation was in M199 at 37 C in a 5% CO_2 atmosphere. Cells in culture were

counted by the method of Pierce, *et al*. (14). Differential cell counts on selected cultures revealed a macrophage purity of greater than 90% after the 3-hour adhesion, and almost 100% after 24-hour culture.

Survival in culture was determined from counts at 24-hour intervals for seven days. Viability was determined by the ability of the cells to exclude a 0.1% solution of Trypan Blue.

Phagocytosis by 24-hr monolayers was determined by the procedure of Tolnai (15), using 1.09-μm latex beads (SIGMA) in a 20:1 bead-to-macrophage ratio.

Pinocytosis by 24-hr monolayers was determined by the method of Steinman (16). After a one-hour incubation in the presence of 0.5 mg/m*l* of horseradish peroxidase (HRP–Sigma), the monolayers were washed and lysed by sonication and the lysates transferred to glass test tubes before being assayed for released HRP. Lysates of

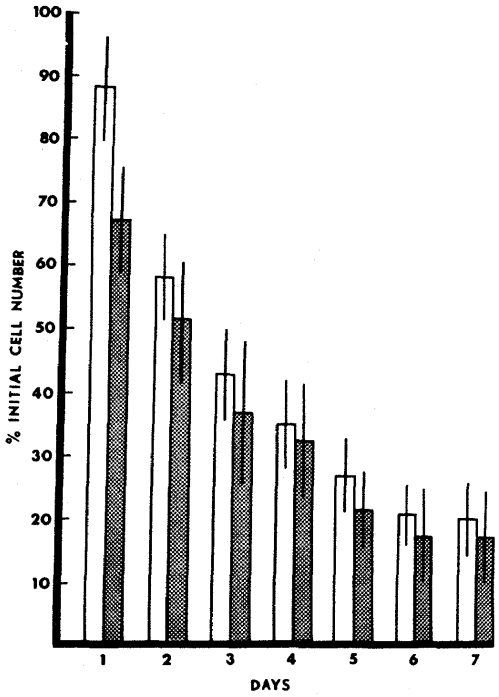

FIGURE 1. Percent viability of alveolar macrophages from inbred A/J mice versus time of *in vitro* culture. "Individual" cultures are denoted by a clear bar which represents the mean and standard error of eight cultures, from eight mice. "Mixed" cultures are denoted by a stippled bar which represents the mean and standard error of four cultures, from eight mice.

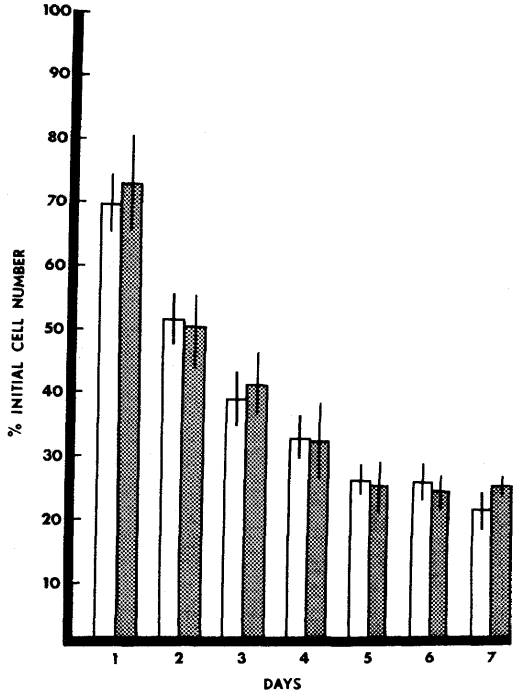

FIGURE 2. Percent viability of peritoneal macrophages from inbred A/J mice versus time of *in vitro* culture. Legend as in Figure 1.

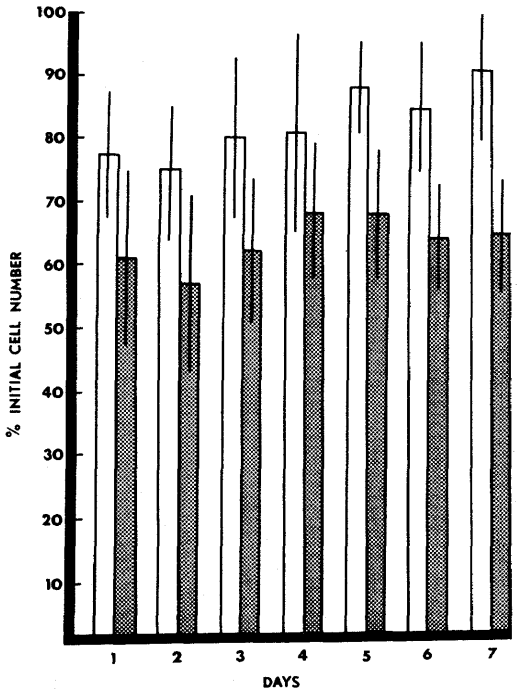

FIGURE 3. Percent viability of alveolar macrophages from Swiss outbred mice versus time of *in vitro* culture. Legend as in Figure 1.

untreated macrophages showed no endogenous peroxidase activity.

Results of individual-vs-mixed cultures were compared using the unpaired Student's t-test, using $p < 0.05$ as the significance level. Graphs represent the mean plus-or-minus standard error-of-the-mean of the data.

RESULTS

Survival in culture of alveolar and peritoneal macrophages from inbred A/J mice is shown in Figures 1 and 2. No differences in survival rates between individual and mixed cultures were found. All days showed the same high correspondence of results ($p > 0.5$) except for day 1 for alveolar macrophages ($0.2 > p > 0.1$).

Survival in culture of alveolar and peritoneal macrophages from outbred Swiss mice is shown in Figures 3 and 4. With the exception of days 5 and 7 for alveolar macrophages, results from individual and mixed cultures showed high correspondence ($p > 0.2$). Days 5 and 7 showed no differences significant at the $p > 0.05$ level ($0.2 > p > 0.1$ and $0.1 > p > 0.05$, respectively). Alveolar macrophages from outbred mice showed no decrease in viability with time, unlike their inbred counterparts. Peritoneal macrophages from these Swiss mice declined in viability as did the cells from inbred mice, but neither as quickly nor as far. Compared to inbred strains, outbred mice commonly yield more macrophages which also survive better in culture (17).

Phagocytosis by peritoneal macrophages is shown in Figures 5 and 6. Alveolar macrophages could not be tested in this or the pinocytosis experiment because of the small yield from individual mice. In neither the inbred nor the outbred mice did mixed cultures differ from individual cultures in percentage of cells phagocytizing particles ($p > 0.5$ in all experiments).

The amounts of horseradish peroxidase pinocytized by peritoneal macrophages are summarized in Figure 7. No consistent or

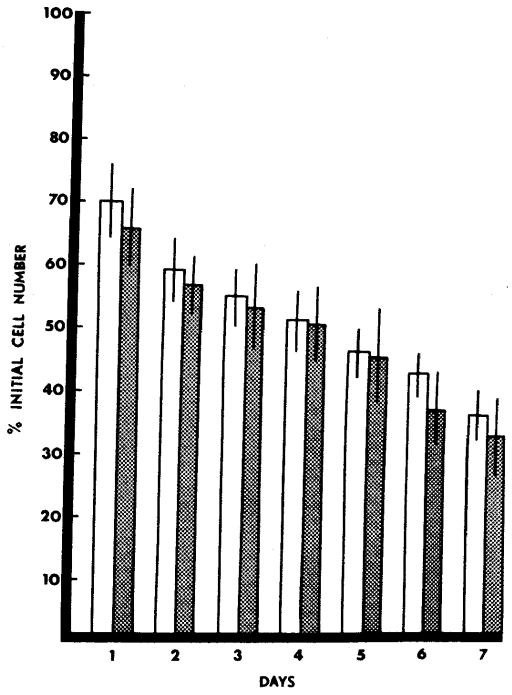

FIGURE 4. Percent viability of peritoneal macrophages from Swiss outbred mice versus time of *in vitro* culture. Legend as in Figure 1.

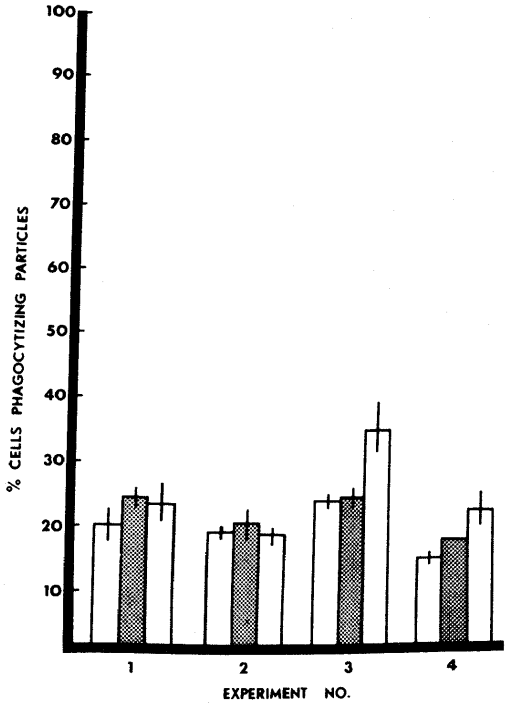

FIGURE 5. Percentage of A/J mouse peritoneal macrophages phagocytizing latex particles. "Individual" cultures are denoted by a clear bar, "mixed" cultures are denoted by a stippled bar. Each bar represents the mean and standard deviation of triplicate cultures from a single mouse. The mixed culture in each experiment was composed from the two individual cultures flanking it. Separate repetitions are denoted by the experiment number.

significant difference in pinocytosis was found between mixed and individual cultures. Cells of outbred mice (Fig. 7A) pinocytized somewhat less than did inbred mice (Fig. 7B). In experiment number 2 using outbred mice (Fig. 7A), the high mean value and large standard error for the mixed culture was due to only one value that was inordinately high. Other values equaled the individual culture pinocytic rates.

DISCUSSION

The pooling of non-induced mouse macrophages did not alter their overt functional activities of survival, phagocytosis, or pinocytosis. The absence of different reactions would seem to indicate that there is no role played by lymphocytes under these culture conditions. This absence of lymphocyte activity could be due to the reported suppressive effects of high macrophage: lymphocyte ratios (18, 19, 20, 21). However, Wing and Remington have recently reported that even when normal macrophages comprise 66-75% of the cell population, lymphocyte stimulation is uninhibited (22). They criticized the former work for using macrophages elicited by glycogen, mineral oil, or thioglycolate – therefore not "normal" – and for having high percentages (up to 30%) of contaminating cell types in their macrophage preparations. Alveolar macrophages, though, definitely suppress lymphocyte activation (23). An alternate explanation for the absence of lymphocyte activity is simply their low numbers in culture. Even though lymphocytes may not have been completely eliminated from the macrophage monolayers, their numbers could be reduced far enough to prevent their reacting with one another or to dilute their effects to inconsequential levels.

The failure of a macrophage-to-macrophage stimulus to affect activity in mixed cultures can be explained, in part, by the manner in which they were harvested. Macrophages collected from the peritoneal cavities of animals previously injected with an exudate-inducing agent have been shown to differ significantly from the normal population (24, 25). Such treatment arti-

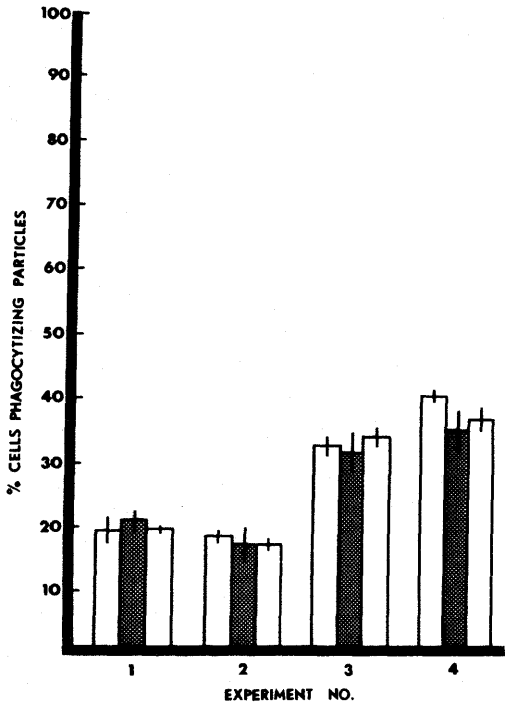

FIGURE 6. Percentage of Swiss mouse peritoneal macrophages phagocytizing latex particles. Legend as in Figure 5.

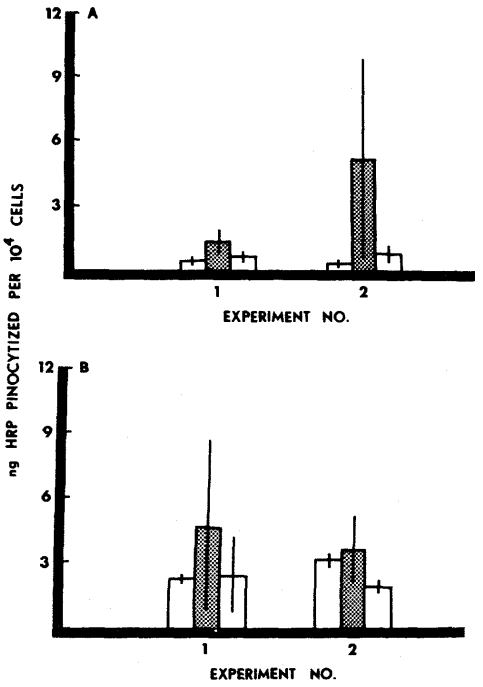

FIGURE 7. Pinocytosis of horseradish peroxidase (HRP) by peritoneal macrophages from: A) Swiss outbred mice, and B) A/J mice. Legend as in Figure 5.

ficially activates macrophages, thus altering their response in culture. For instance, it has been shown that macrophages activated by such harvesting procedures display stronger cytostatic effetcs (7, 26).

There exists an important caveat to the above conclusions indicating that there is no effect of pooling macrophages. In both inbred and outbred mice, mixed cultures of macrophages showed a greater variability in their pinocytic rates than did cultures from individual animals. This variation could conceivably denote a type of mixed macrophage reaction. However, the variation was most pronounced in only one of the two experiments using either inbred or outbred mice. Therefore, multiple trials should expose anomalous results.

The results obtained using these three criteria lend increased trust that experiments using pooled cells will yield data equivalent to those that can be obtained using individual mice.

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