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Antigen Competition: A Paradox

Abstract. *Immunization of mice with pig erythrocytes caused impairment of the antibody response to subsequent immunization with sheep erythrocytes, a phenomenon called "antigen competition." Paradoxically, spleen cells from mice previously injected with pig erythrocytes produced an* increased *response when immunized in vitro with sheep erythrocytes. Augmentation of the in vitro response is due to an increase in one of the interacting cell types. "Antigen competition" is not due to competition for cells. Cell transfer experiments provided evidence that "antigen competition" observed in animals is the result of a humoral factor, presumably antibody, present in the animal but eliminated during preparation of cells for culture.*

The phenomenon of "antigen competition," that is, when the antibody response to one antigen is reduced by a prior injection of a second unrelated antigen, is observed in many species with a wide variety of antigens (*1*). However, no explanation of the mechanism of this phenomenon has been satisfactory. Two alternative hypotheses have been proposed, each with its own implications for present theories of antibody formation. According to one hypothesis, competition occurs for a multipotential stem cell, partial exhaustion of which reduces the response to the second antigen (*2, 3*). According to an alternative hypothesis, suppression is mediated by a humoral factor. A humoral factor required for the response to both antigens could be exhausted by stimulation with the first antigen. Alternatively, a humoral factor produced in response to the first antigen could suppress the response to the second antigen (*4*). Below, evidence is presented that spleen cells obtained from mice which would demonstrate the phenomenon of "antigen competition" are capable in vitro of an augmented response to a second antigen. This augmented in vitro response is the result of an increase in the numbers of at least one of the several cell types required for the in vitro immune response. "Antigen competition," that is, the suppression observed in vitro, is apparently due to a humoral factor, probably antibody.

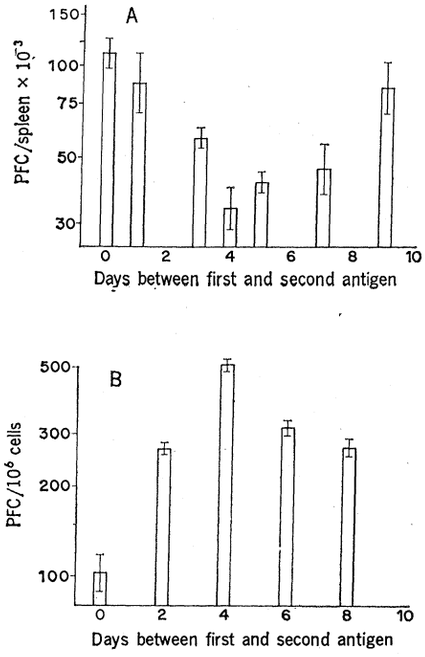

Fig. 1. (A) The in vivo 4-day plaque-forming-cell (PFC) response of mice immunized with sheep erythrocytes at various intervals after injection of pig erythrocytes. (B) The in vitro PFC response of spleen cell cultures immunized with sheep erythrocytes at various intervals after in vivo immunization with pig erythrocytes. Each bar represents the average of at least three animals or three cultures, respectively. The standard error of the mean is indicated.

DBA mice, 7 to 12 weeks old, were used. Pig erythrocytes and sheep erythrocytes were chosen as the two antigens because of their low reciprocal cross-reactivity (*4*). The response to these erythrocytes was assayed by the direct hemolysis-in-gel technique (*5*).

When 10^8 sheep erythrocytes were given intravenously 4 days after an injection of 5×10^8 pig erythrocytes, the 4-day plaque-forming-cell (PFC) response to sheep erythrocytes was suppressed about fivefold (Fig. 1A). With longer or shorter intervals between injection of the two antigens, suppression was less. When the two antigens were injected simultaneously, there was no suppresson of response to either antigen. These results are similar to those of Radovich and Talmage (*4*).

An augmented PFC response to sheep erythrocytes was observed, however, when spleen cells from animals immunized 2, 4, 6, or 8 days previously with pig erythrocytes were immunized in vitro with sheep erythrocytes. The culture method of Mishell and Dutton was used with 10^7 spleen cells per culture, and 10^7 sheep erythrocytes as antigen (*6*). The PFC response to sheep erythrocytes was measured at 4 days. The prior in vivo exposure to pig erythrocytes caused an increase rather than a decrease in the in vitro response to sheep erythrocytes (Fig. 1B). The maximum increase in

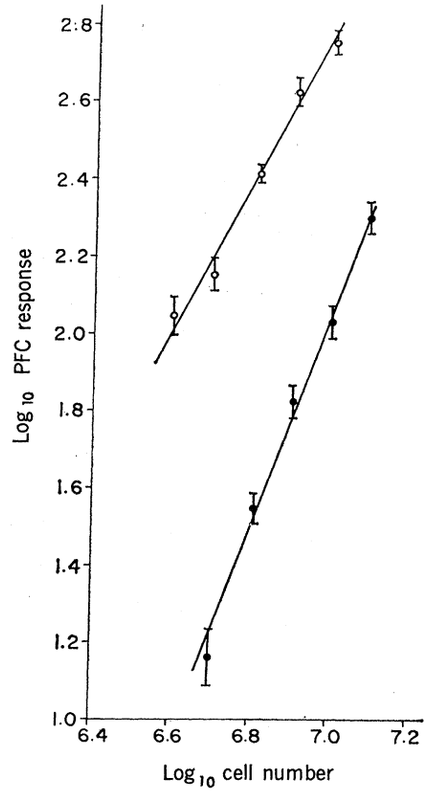

Fig. 2. The $\log_{10}$ of the plaque-forming-cell response versus the $\log_{10}$ of various numbers of spleen cells immunized in culture with sheep erythrocytes: normal cells (●) and cells from mice injected with pig erythrocytes 4 days prior to culture (○). By linear regression analysis the slope of the response of normal cells = 2.83 ± 0.16; the slope of pig erythrocyte–primed cells = 1.97 ± 0.10. The slopes are significantly different ($P < .01$).

the response occurred when the interval between in vivo immunization with pig erythrocytes and the initiation of cultures was 4 days. At longer or shorter intervals the augmentation was less. Thus, spleen cells from mice that would have shown a decreased response in vivo were capable of an increased response in culture. Similar results were obtained by using horse erythrocytes, burro erythrocytes, or *Bordetella pertussis* vaccine as the first antigen and sheep erythrocytes as the second.

Two types of experiments were performed to determine the basis for the enhanced in vitro response. First, on days 2, 3, and 4 of culture, the PFC response of normal spleen cells to sheep erythrocytes was compared with the PFC response to sheep erythrocytes of spleen cells from mice primed with pig erythrocytes. The responses were expressed as exponentials, and slopes of the response lines, reflecting the rate of increase of PFC, were not significantly different (0.89 ± 0.08 $\log_{10}$ unit per day versus 0.95 ± 0.07). The response of spleen cells from pig erythrocyte–immune mice was about fivefold greater throughout. The increased response was therefore due to an increased number of initially responding cells.

Second, spleen cells from normal mice and mice primed with pig erythrocytes were cultured at various dilutions, and the response to sheep erythrocytes was measured at 4 days. The initial cell number per culture and the response were expressed as logarithms, and linear regression analysis was used to determine the line of best fit. Assuming initial random interactions, the slope of the line reflects the minimum interactions required between cell types in limiting numbers (*7*). Interpreted in this way, these data (Fig. 2) indicated that prior immunization with pig erythrocytes caused an increase in the number of one of the cell types necessary for the in vitro response to sheep erythrocytes.

Spleen cells can be separated into two populations, one relatively adherent to plastic, the other nonadherent, both of which are required for the in vitro immune response (*8*). Experiments involving reciprocal recombinations of adherent and nonadherent cell populations derived from spleens of normal or pig erythrocyte–immunized mice showed that the nonadherent population accounts for the augmented response of pig erythrocyte–immune spleen cells. Conversely, adherent populations derived from normal or pig erythrocyte–immune spleen cells did not differ in effectiveness. The nonadherent population contains both bone marrow–derived cells, which synthesize antibody, and thymus-derived cells (*9*). At present it is unknown which of these cell types may be increased. However, the increased response to sheep erythrocytes does not appear to result from an increase in the number of cells producing hemolytic antibody to antigens shared by pig and sheep erythrocytes. This conclusion is based on the observation that cultures of spleen cells from animals previously immunized with pig erythrocytes had 224 ± 13 PFC per 10^6 cells at the initiation of culture, but only 7 ± 2 PFC per 10^6 cells at 4 days. In the same cultures the number of PFC against sheep erythrocytes rose from 5 ± 2 initially to 988 ± 72 PFC per 10^6 cultured cells at 4 days. The 4-day response of normal cells to sheep erythrocytes was only 151 ± 22 PFC per 10^6 cells cultured. Thus the cells producing hemolytic antibody to antigens shared by both erythrocytes can account for only a small fraction of the increased response observed in culture.

Table 1. The plaque-forming-cell (PFC) response to sheep erythrocytes, at 6 days, of 10^8 spleen cells from normal mice transferred to syngeneic recipients previously irradiated with 850 r. Pig erythrocyte–primed recipients received pig erythrocytes 4 days prior to irradiation. Normal recipients received no prior treatment. Each response represents the average of at least four animals. The number in parentheses represents the logarithm_{10} of the mean $\pm$ the standard error of the mean. The average of the difference between the means of each experiment was significantly different from 0 ($P < .01$).

Experiment	Ratio, PFC/spleen cells	
	Normal recipients	Pig erythrocyte–primed recipients
1	3750 (3.57 ± 0.22)	1250 (3.10 ± 0.28)
2	510 (2.71 ± 0.29)	180 (2.25 ± 0.26)
3	330 (2.52 ± 0.26)	140 (2.14 ± 0.28)

These observations demonstrate that spleen cells from an animal that would show an impaired response to a second antigen have an augmented response to the second antigen in culture. Clearly, the phenomenon of "antigen competition" observed here cannot be explained by exhaustion of a cell population, but must be determined by factors present in vivo which are not present in vitro. The existence of a humoral factor is indicated by spleen cell transfer experiments. Normal spleen cells were transferred to heavily irradiated (850 r) mice, either untreated mice or mice which had been injected with pig erythrocytes 4 days previously. The recipient mice were then immunized with sheep erythrocytes and their spleens were assayed for PFC to sheep erythrocytes 6 days later. In three consecutive experiments the response of the recipients previously injected with pig erythrocytes was lower than the response of control mice (Table 1). These results are consistent with a humoral factor present in mice previously immunized with pig erythrocytes which suppressed the response of normal spleen cells to sheep erythrocytes, and are in agreement with findings recently reported (*10*).

Such a humoral factor could be an antibody directed against sheep erythrocytes. The number of PFC against sheep erythrocytes after pig erythrocyte injection increases slightly and reaches a maximum of about 500 PFC at 4 days, but no serum antibody is detectable by conventional titer methods. Passively administered antibody in less than detectable amounts can cause specific suppression, probably by reducing the number of cells responding an antigenic stimulus (*11*). The conclusion of Albright *et al.* that the number of responding cells was reduced by antigen competition could be explained by this inhibitory action of antibody (*2*). This hypothesis is also consistent with the findings of Hanna and Peters that antigen competition results in an impairment ultimately affecting the immunocompetent unit reflected by a suppressed secondary as well as primary antibody response (*12*).

Spleen cells obtained from mice which would have had a decreased response in vivo because of prior exposure to the first antigen are capable of an augmented response in culture to the second antigen. These paradoxical findings can be resolved as follows. The first antigen injection generates a small increase in the numbers of cells reacting against the second antigen. In vivo the antibody product of these cells results in a suppressed response, while the augmented response in vitro reflects the increase in the number of responding cells. Studies of the phenomenon of "antigen competition" should yield information about the control mechanisms of the immune response, and about the nature of the interactions among the various cell types involved.

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Neuraminidase Activity in HeLa Cells: Effect of Hydrocortisone

Abstract. *Homogenates of HeLa cells contain neuraminidase activity. This enzyme is particle-bound, and it has a* p*H optimum of 4.2. Hydrocortisone-regulated cells contain two to three times as much neuraminidase as the corresponding controls. The hydrocortisone treatment also causes an increase in the cell content of β-glucuronidase and acid deoxyribonuclease.*

The regulation of glycoprotein turnover in dividing cells underlies important aspects of cell biology due, in part, to the known involvement of these compounds in the biochemistry of cell membranes and in the determination of the immunological characteristics of the cells. The HeLa cells cultured in the presence of added hydrocortisone contain more sialic acid than do their corresponding untreated controls (*1*). We have now established the presence of a particle-bound neuraminidase (E.C. 3.2.1.18) in $HeLa_{71}$ cells, and we have studied some properties of this enzyme and the effects of hydrocortisone on the enzyme activity.

The $HeLa_{71}$ cells (*2*) were grown in monolayer culture with Eagle's minimum essential medium supplemented by calf serum (10 percent), penicillin (50 unit/ml), streptomycin (50 μg/ml), and kanamycin (50 μg/ml). The hydrocortisone-regulated (Hcr) cells were obtained by subculturing the cells in a medium containing 1.0 μg of hydrocortisone per milliliter for a minimum of 1 month. The Hcr state is characterized by a new steady-state level of growth and a new cellular physiological constitution (*3*). The cells, grown in monolayers at 37°C, were harvested from the Blake bottles near the end of exponential growth (about 72 hours) by gentle scraping into 10 ml of saline, buffered with 5 m*M* tris(hydroxymethyl)-aminomethane-hydrochloride (tris-HCl) (*p*H 7.4). The cells were washed twice in the same buffered saline, and a small sample was taken for cell counting in a hemocytometer. The cells were packed by centrifugation, and the pellet was suspended in ice-cold RSB buffer (10 m*M* NaCl, 5 m*M* $MgCl_2$, and 10 m*M* tris-HCl; *p*H 7.4). The suspension (about 1 ml per bottle) was homogenized in a tight-fitting, all-glass Dounce homogenizer. Complete homogenization was obtained after ten strokes, and the thoroughness of the homogenization was checked by phase-contract microscopic examination. The homogenate was centrifuged for 10 minutes at 700*g* to sediment the nuclear fraction, and the supernatant fraction was decanted with a Pasteur pipette. These cytoplasmic extracts, which gave very low blank values during the neuraminidase assay, were utilized for the studies of the properties of this enzyme. The enzyme preparations consisted of nine volumes of cytoplasmic extract and one volume of 1*M* sodium acetate–acetic acid buffer.

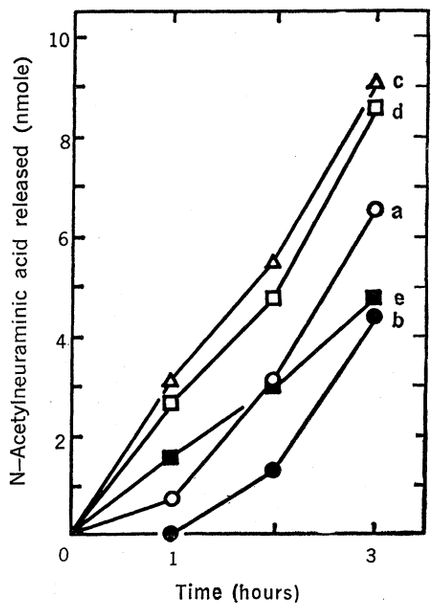

Fig. 1. Progress curves of $HeLa_{71}$ neuraminidase. (*a*) Control preparation; (*b*) control preparation subjected to ultrasonic treatment. The other curves represent control preparations assayed in the presence of Triton X-100 at the following concentrations (in percent by volume): (*c*) 0.02, (*d*) 0.04, and (*e*) 0.10.

For the neuraminidase assay (*4*), 400 nmole of neuramin-lactose (*5*) dissolved in 0.1 ml of distilled water was added to 0.3 ml of enzyme, and the mixture was incubated for 3 hours at 37°C. The reaction was stopped by adding 0.2 ml of periodate reagent (*6*), and the free *N*-acetylneuraminic acid was determined by the thiobarbituric acid reaction of Warren (*6*). The neuraminidase from preparations of control and Hcr cells showed essentially identical properties. The progress curve (Fig. 1) showed a nonlinear response, that is, a low rate of hydrolysis for the first hour, followed by an apparent activation and linear response afterward. Addition of low concentrations of Triton X-100 resulted in linear response (Fig. 1) and higher activity. The optimum conditions were obtained with a final concentration of detergent between 0.02 and 0.04 percent (by volume). Higher concentrations of Triton X-100 caused increasing inhibition of the enzyme (Fig. 1). A marked inhibition of the neuraminidase activity occurred when the cytoplasmic extract was subjected to a 2-second burst of ultrasound delivered by the microprobe of a Branson Bio-Sonicator at 80 percent efficiency with a 14-kc output (Fig. 1). The *p*H optimum was about 4.2. The activities between *p*H 4.0 and 4.4 were essentially identical and decreased gradually below *p*H 3.8 and above *p*H 4.6. At *p*H 5.8 only 20 percent of the maximum activity was detected. The supernatant fraction of homogenates centrifuged for 1 hour at 105,000*g* showed no neuraminidase activity when assayed either at *p*H 4.2