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Effect of Temperature on Suppressor Cells in Chicken Spleen Cell Cultures[1] (41891)

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Abstract. The plaque forming cell (PFC) response of *in vivo* primed chicken spleen cells to sheep erythrocytes (SRBC) at 37°C *in vitro* was much higher when antigen was added on Day 2 of culture than on Day 0, at the initiation of the incubation period. No such difference was observed when the cells were cultured at 40°C, in which case both responses were low. As shown previously the effect of delayed exposure to SRBC at 37°C was due to a disappearance of suppressor T cells in the absence of antigen, which did not occur at 40°C. Addition of concanavalin A on Day 0 could, even in the absence of SRBC, maintain the suppressor cell activity at 37°C. The results suggest that suppressor cell activity is very temperature dependent.

In a previous report it was shown that proliferative responses and mixed lymphocyte reactions of chicken spleen or peripheral blood cells were much higher at 40–41°C than at 37°C (1). However, antibody production to sheep red blood cells (SRBC) was at least as good in primed spleen cells reexposed to antigen at 37°C as compared to 40°C (2, 3). An important aspect of the response to SRBC at 37°C *in vitro* was that it was much higher when antigen was added 2 days after initiation of culture than when it was added immediately after explantation of the spleen cells. This effect was found to be due to the disappearance, over the first 1–2 days *in vitro,* of antigen-specific suppressor T cells which could be kept active in the presence, but not in the absence of antigen. On the other hand, helper T cells remained active and were responsive to antigen added after 2 days (3).

In the present study we have examined the antibody response at 40°C in more detail. It will be shown that suppressor cells survive in spleen cells cultured without antigen at 40°C although they disappear at 37°C. This appears to be the reason that antibody production is as good or even better at 37°C than at 40°C, depending on the time of antigen addition.

Materials and Methods. *Animal immunization.* Chickens of strain FP (B15/B21) were purchased as eggs from Hy-Line International (Dallas Center, Iowa). Eggs were incubated and hatched in a Petersime Model 5 incubator–hatcher (Petersime Incubator Co., Gettysburg, Ohio). Chickens of 4 months of age or older were injected iv with 0.2 ml of 5–20% SRBC. Their spleens were removed for culture 2 days after antigen injection.

Cell culture. Petri-dish cultures were prepared as described previously (3), using a modification of the method of Mishell and Dutton (4) for mouse cells. Antigen-primed spleen cells ($1–1.2 \times 10^7$/dish) were cultured in 1 ml of modified minimum essential medium with 5% agammaglobulinemic ($A\gamma$) chicken serum, kept in a 10% CO_2 + 7% O_2 atmosphere and fed daily with nutrient cocktail, containing vitamins, amino acids, and bicarbonate as described (4) but no fetal calf serum. In cell-mixing experiments, target cells were precultured without antigen for 2 days before mixing with putative "suppressor cells" and antigen. In most cultures 50 μl of 2% SRBC were used as the antigen. In some cultures SRBC previously treated for 20 hr with 0.2% glutaraldehyde were used.

Plaque forming cell assay. The anti-SRBC response was expressed as PFC per dish on Day 4 of culture. PFC were detected by a slide modification of the Jerne plaque assay (4, 5). During the last 45 min of culture prior to assay, 0.1 ml fresh normal chicken serum, usually combined with 0.1 ml guinea pig C, was added per milliliter of culture in order to lyse any antibody-coated SRBC remaining in the cultures which might otherwise lead to

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TABLE I. EFFECT OF TEMPERATURE ON SECONDARY PFC RESPONSES TO SRBC BY SPLEEN CELLS *In Vitro*

SRBC added on Day	Indirect PFC/dish[a]									
	Expt 1		Expt 2		Expt 3		Expt 4		Expt 5	
	37°C	40°C	37°C	40°C	37°C	40°C	37°C	40°C	37°C	40°C
—	109	41	187	103	270	162	417	412	72	45
0	300	32	455	285	1565	1535	2400	2015	256	168
2	2386	268	2935	600	5320	1760	8617	2515	4100	208
0 + 2	177	45	583	340	ND	ND	ND	ND	ND	ND

[a] Mean of two to four cultures; culture period of 4 days. Spleen cells taken from FP chickens injected iv 2 days earlier with 0.2 ml of 5% SRBC. Cultures were maintained 4 days and received 50 μl 2% SRBC on Day 0 and/or Day 2.

artifactual plaque formation (6). The cells were harvested, washed twice, counted, and resuspended to 1 or 2 ml per dish. Cells were plated using 2–8 × 10^5 cells per slide. For PFC development, slides were flooded with a 10% solution of guinea pig C (absorbed with SRBC) containing rabbit anti-chicken Ig (1:1000), and incubated for 45 min at 37°C. The average value of PFC from duplicate cultures which had numbers not differing >10% were recorded. PFC obtained in the absence of antigen were always determined.

Results. The data in Table I show that spleen cells taken 2 days after priming with SRBC iv and cultured at 37°C responded much better when the antigen was added on Day 2 than on Day 0 of culture. The mean ratio of the magnitudes of PFC responses per dish after addition of antigen on Day 2 as compared to Day 0 was 7.5 at 37°C, as opposed to a mean ratio of 2.8 at 40°C. When antigen was given on both days the results were exactly like those where antigen was given on Day 0 alone. The responses at 40°C were at the lower level regardless of when the antigen was added.

Since the spleen cells were taken from primed chickens they might continue to make antibody *in vitro* particularly at 40°C even without further antigen addition. Thus, it seemed possible that destruction of the SRBC immediately after addition on Day 2 to the cultures at 40°C caused the lack of response in these cultures. We, therefore, repeated the experiments using glutaraldehyde-fixed SRBC as the antigen. The results in Table II show that such pretreatment of the SRBC in no way affected the results. The glutaraldehyde-fixed SRBC were resistant to lysis and these results therefore also exclude any influence of artifacts due to the lysis of agglutinated SRBC in the PFC assay on the magnitude of the PFC responses.

The survival of the cultured cells at the two different temperatures was very similar. In a typical experiment in which 1.2 × 10^7 cells

TABLE II. EFFECT OF TEMPERATURE ON PFC RESPONSE TO SRBC IS ALSO SEEN WITH GLUTARALDEHYDE-FIXED ANTIGEN

SRBC added		Indirect PFC/dish on Day 4			
		Expt 1		Expt 2	
Pretreatment	Day of culture	37°C	40°C	37°C	40°C
—	—	260	200	470	430
Untreated	0	1690	1560	2340	1880
Untreated	2	5550	1380	8390	1850
Glutaraldehyde treated[a]	0	1430	1510	2460	2150
Glutaraldehyde treated[a]	2	5090	2130	8850	3180

[a] 50 μl of 2% SRBC. Incubated for 20 hr with 0.2% glutaraldehyde prior to use.

TABLE III. LOSS OF SUPPRESSOR CELLS BY PREINCUBATION WITHOUT ANTIGEN AT 37° BUT NOT AT 40°C

Suppressor cells added on Day 2[a] (precultured for 2 days)	Indirect PFC/dish[b] on Day 4
Without SE at 37°C	7025
With SE at 37°C	362
Without SE at 40°C	654
With SE at 40°C	692
No suppressor cells added	4100

[a] Putative "suppressor cells" (5×10^6) were added to dishes containing 10^7 "target" Day 2 immune spleen cells which had been precultured for 2 days without antigen at 37°C. Antigen was added and cultures continued for an additional 2 days. Suppressor cells were taken from the same chicken as target cells and precultured as indicated.

[b] PFC/dish, mean of duplicate cultures.

were cultured per dish, all dishes harvested on Days 2 to 4 contained 7–8 $\times$ 10^6 viable cells, regardless of the temperature at which they had been cultured or of the time of antigen addition.

As previously shown (3), cells precultured for 2 days at 37°C without antigen can be used as targets for suppression, since their own suppressor cells have disappeared. Thus, we previously showed that T cells kept for the first 2 days in culture at 37°C with antigen suppressed the higher responses of cells given antigen on Day 2. The results in Table III show that this suppressive effect was also shown with spleen cells precultured with antigen for 2 days at 40°C. When cells were precultured without antigen at 37°C, they did not cause the suppression, as shown previously (3), but cells precultured without antigen at 40°C still did (Table III). Supernatants taken from spleen cells cultured with antigen, either at 37°C or at 40°C, did not cause such suppression (data not shown). These findings suggest that the responsiveness to antigen at 40°C increases very little after preculture for 2 days without antigen, because the majority of the suppressor cells survive at this temperature even in the absence of antigen, whereas they do not at 37°C.

When spleen cells were precultured without antigen at 37°C for 2 days, and then moved to 40°C prior to challenge with antigen, they responded as well as did cells given antigen on Day 2 of culture and kept at 37°C. This finding showed that, once the suppressor cells had functionally disappeared by culture at 37°C in absence of antigen, they could not be regenerated by increasing the culture temperature to 40°C (Table IV). On the other hand suppressor cells, induced at 37°C by addition of antigen immediately after explantation of the spleen cells, continued to suppress the response when the cultures were moved to 40°C. If the first 2 days of culture were at 40°C, regardless of the presence of antigen, the cells could not be made to give high anti-SRBC responses even when they were then moved to 37°C. In addition, cells cultured without antigen but in the presence of concanavalin A (Con A) during the first 2 days at 37°C, failed to give high PFC responses to

TABLE IV. TEMPERATURE DURING FIRST INCUBATION PERIOD DETERMINES RESPONSIVENESS TO ANTIGEN ON DAY 2

		First 2-day culture period at 37°C		First 2-day culture period at 40°C	
Antigen added (Day)	Second culture period (°C)	Expt 1 ΔPFC/dish[b]	Expt 2 ΔPFC/dish[b]	Expt 1 ΔPFC/dish[b]	Expt 2 ΔPFC/dish[b]
2	37	1438	6608	424	1904
0	37	204	681	320	341
2	40	1646	7358		979
0	40	310	866		787
2[a]	37		583		
0[a]	37		521		

[a] Con A (10 μg) added to cultures on Day 0.

[b] Mean of duplicate dishes, determined on Day 4. PFC/dish in absence of antigen were 70 (Expt 1) and 142 (Expt 2), and were subtracted from the values in the table.

antigen added on Day 2, presumably because Con A stimulated the survival of suppressor cells in an antigen nonspecific manner as had previously also been shown with pokeweed mitogen (3).

Discussion. The present results show that, in the absence of SRBC, the survival of suppressor cells in cultures at 40°C, the normal body temperature of the chicken, is better than at 37°C. As in our previous studies (3), the activity of suppressor T cells could be maintained at 37°C by addition of the sensitizing (but not of unrelated) antigen, which resulted in much lower responses upon immediate than upon delayed addition of the antigen to the cultures. No such loss of suppressor cells can be seen at 40°C with the paradoxical result that PFC responses *in vitro* are lower at the higher temperature, particularly upon delayed addition of antigen.

Although it is difficult to relate findings with cells from cold blooded with those from warm blooded vertebrates, it should be mentioned here that cytotoxic responses obtained with shark peripheral blood cells at 30°C are lower than at 23°C *in vitro,* due to the increased activity of suppressor cells at the higher temperature. However, reports on the effect of temperature on the PFC response of mice to SRBC have shown that responses at 39.5°C are higher than at 37°C. This effect is found in spite of greater suppressor T cell activity at the higher temperature. The effect has been attributed to an overall better responsiveness of all cell types (7). The recently described higher sensitivity of murine cells to interleukin-1 at 39°C than at 37°C (8, 9) may be related to this finding.

Temperature changes such as those studied here greatly increase proliferative responses to T-cell mitogens of both peripheral blood and spleen cells of the chicken (1, 10), but an analysis of subpopulations in these responses has not been made in the absence of available cell surface markers for chicken T-cell subsets. The present results demonstrate that Con A stimulation, initiated on Day 0 at 37°C maintains the suppressor cells for the SRBC response, as was also shown previously for pokeweed mitogen (3).

Delayed addition of mitogen (11–14) or of antigen (14, 15) to human peripheral blood cells has been reported to result in greater proliferative responses than addition of these agents immediately upon explantation of the cells. This effect has been attributed to a change in sensitivity of the cells to prostaglandins produced by accessory cells (12, 13, 16) but indomethacin does not in all cases influence the effect of delayed antigen addition (15). Thus the occurrence of a transient suppressor cell in human peripheral blood cell cultures (17) is a well-recognized phenomenon. The present studies suggest that an examination of the effect of temperature on this phenomenon might be of interest.

Since in the absence of specific antigen or mitogen the suppressor cell activity in the chicken spleen cell cultures is greater and/or maintained better at 40°C than at 37°C, it appears that suppressor cells are quite sensitive to temperature changes—in this case hypothermia. However, a temperature-dependent change in sensitivity to regulatory effects of prostaglandins or difference in prostaglandin production could also indirectly mediate the apparent survival as opposed to loss of suppressor cells at 40°C as compared to 37°C.

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