ANTIGEN-INDUCED CHANGES IN LYMPHOID CELL HISTONES

II. Regional Lymph Nodes

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ABSTRACT

The nuclear histones of control and antigen-affected lymphocytes and thymocytes were measured in terms of their intensity of staining with ammoniacal-silver (A-S) and alkaline fast green (FG). Subcutaneous injection of full strength and doubly concentrated tetanus toxoid into the upper forelimb was followed by an acute decrease in the intensity of the FG and A-S staining of the thymocyte nuclear histones. Such changes in staining were delayed in the nuclear histones of the regional axillary lymph nodes. In contrast, the injection of a 1:100 dilution of tetanus toxoid caused an acute decrease in staining of the nuclei of the regional lymph node lymphocytes but only minimal changes in the thymocytes. An acute decrease in staining of the lymph node lymphocytes also occurred when heterologous serum proteins were used as the antigen. Prior administration of cortisone was found to inhibit the antigen-induced changes in the staining of lymphocyte nuclear histones. No such inhibitions occurred after the administration of saline solution. The findings suggest that the nuclear histones play a significant role in the immunological competence of lymphoid cells. However, additional studies are needed to define the biochemical basis of the observed changes as well as the mechanisms whereby antigen-induced changes in nuclear histones participate in the complex cellular and serological alterations which are evoked by antigens, *viz.* plasma cell formation and antibody synthesis.

In a previous study we found that the injection of an antigen causes an acute decrease in the intensity of staining of nuclear histones of thymocytes with ammoniacal-silver (A-S) or alkaline fast green (FG) (1). The present study is principally concerned with the histones of lymphocytes of lymph nodes draining the site of an antigen injection. Observations have also been made on the action of cortisone on antigen-induced changes in the histones of lymphoid cells.

MATERIALS AND METHODS

The present study utilized male and female CF-1 mice (Carworth Farms, New City, New York) weighing 15 to 20 gm. The principal antigen employed was tetanus toxoid (Wyeth) twice concentrated, full strength, and diluted 1:100 with sterile physiological saline. One-tenth of a milliliter of the appropriate concentration of tetanus toxoid was injected subcutaneously into the medial aspect of the upper forelimb. The injected mice were sacrificed at various times after the injection (30 minutes, 1 hour, 2 hours, and 24 hours). Smears were then prepared of the thymus gland and the ipsilateral axillary lymph nodes. In similar studies human serum and commercial preparations of 16.5 per cent solution of human gamma globulin were used as the antigen.

The effect of cortisone was tested by injecting 0.1 ml (2.5 mg) of a cortisone acetate suspension into one

TABLE I

Sequential Changes in Ammoniacal-Silver Staining of Lymphoid Cells after the Administration of Diluted and Concentrated Tetanus Toxoid

	Injected lymph node		Thymus	
	1:100	Concentrated	1:100	Concentrated
Control	1.02 ± 0.01		0.98 ± 0.02	
30 minutes	0.94 ± 0.02	0.99 ± 0.01	0.78 ± 0.01	0.70 ± 0.01
1 hour	0.64 ± 0.01	0.83 ± 0.02	0.82 ± 0.01	0.60 ± 0.02
2 hours	0.69 ± 0.01	0.83 ± 0.02	0.76 ± 0.01	0.58 ± 0.02
5 hours	0.82 ± 0.02	0.75 ± 0.02	0.82 ± 0.02	0.79 ± 0.01
24 hours	0.77 ± 0.02	0.83 ± 0.02	0.82 ± 0.01	0.79 ± 0.01

forelimb and a like amount of 0.9 per cent sodium chloride solution into the other forelimb of the same mouse. Fifteen minutes later a 0.1-ml dose of full strength tetanus toxoid was injected into both forelimbs. Two hours after the toxoid injection the mouse was sacrificed and smears were prepared from the axillary lymph nodes of both sides.

All the smears were air dried at room temperature, fixed overnight in 10 per cent sodium acetate-neutralized formalin, and stained for histones by the fast green (FG) method of Alfert and Geschwind (2) and by the ammoniacal-silver (A-S) method developed in this laboratory (1, 3–5), and for DNA by the Feulgen procedure. The stained nuclei of small lymphocytes and mature thymocytes were then measured cytophotometrically by means of a Canalco cytophotometer as previously described (1), except that red-sensitive R-136 phototubes, kindly provided by Dr. Leonard Ornstein of Mt. Sinai Hospital, New York City, were used instead of 1-P-21. Measurements of the extinction values of the A-S–stained nuclei were made at 480 mμ (E480) and at 700 mμ (E700). As reported previously, the E480 represents the total A-S–stainable histone, and the E700 represents the black-staining histone fraction, which is low in lysine. The samples measured from each slide included 20 to 40 nuclei, selected for spherical undistorted shape.

A number of the representative smears were also doubly stained with A-S and the Feulgen procedure. Such preparations were measured at 440, 560, and 700 mμ. The A-S–Feulgen (AS-F) staining method provides a measure of the intensity of the A-S staining relative to the intensity of the Feulgen staining of DNA in the same nucleus.

RESULTS

Measurements of the Feulgen staining of the lymphocytes and thymocytes revealed no difference between the control and antigen-treated cells within the period of observation. The mean relative amount of DNA per lymphocyte in the control and the antigen-treated group was 0.395 ± 0.003 and 0.406 ± 0.003, respectively, as determined from preparations stained with the Feulgen procedure above. Approximately the same amount was found for thymocyte nuclei, control and antigen-treated, prepared and stained at the same time and in the same way. In contrast to this constancy of DNA, the histone content, as determined by A-S and FG staining of the thymocytes and lymphocytes, varied in relation to the concentration of the antigen and the time elapsed after injection. The details of such changes in histone staining were as follows.

Axillary Lymph Nodes

DILUTED TETANUS TOXOID: The acute effects of the subcutaneous injections of various amounts of tetanus toxoid on the staining of nuclear histones of the small lymphoid cells of the proximal lymph node and the thymus are given in Table I and Figs. 1 and 2. As indicated therein, the injection of the 1:100 dilution of tetanus toxoid was followed by an acute decrease in the A-S staining of the nuclear histone of the local lymph node lymphocytes. This decrease was clearly apparent 1 hour after the injection of the diluted antigen and lasted for 2 hours after the injection. Such quantitative changes were accompanied by a change in the A-S staining of the lymphocytes from a yellowish brown to a black color. However, by 5 to 24 hours after the injection of the dilute antigen the quantitative and qualitative staining approached the preinjection values. As indicated, the decrease in A-S staining 1 hour after the injection of diluted antigen was also accompanied by a decrease in FG staining of the lymphocyte histones.

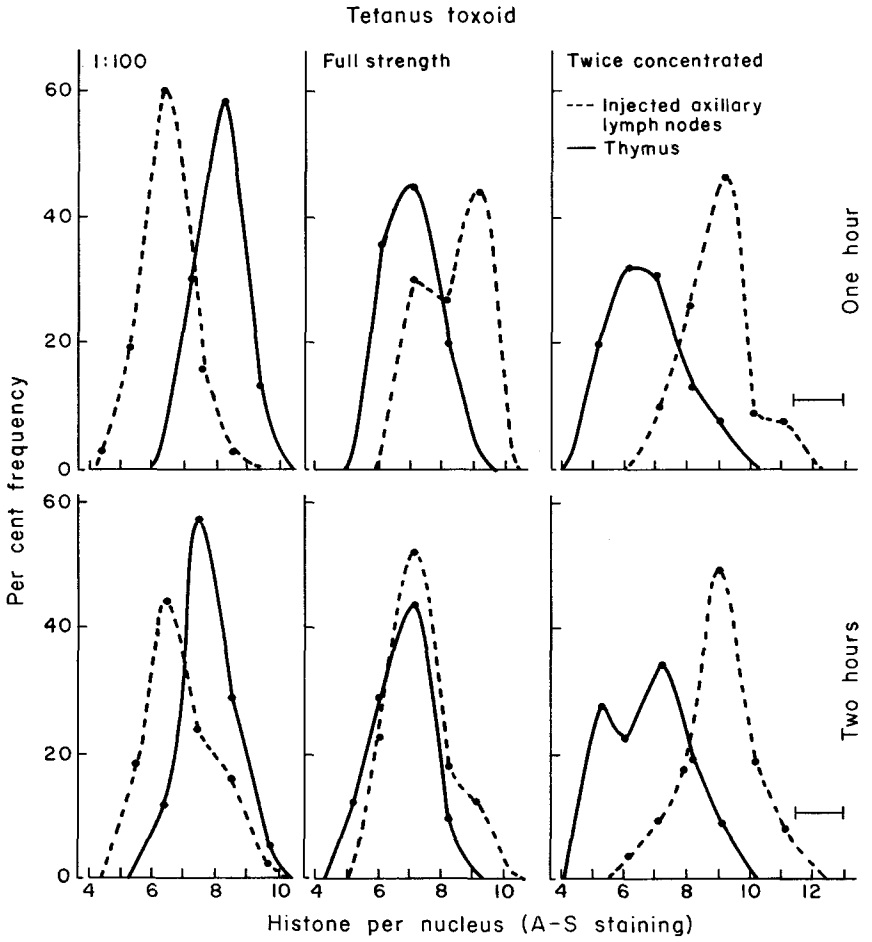

FIGURE 1 Intensity of ammoniacal-silver (A-S) staining of nuclei of thymocytes and regional lymph node lymphocytes 1 hour and 2 hours after the injection of different concentrations of tetanus toxoid into the upper forelimb. The diluted antigen produces a more acute decrease in the stainability of lymphocyte histone than do the full strength and concentrated antigens. In contrast, the thymocyte histones are more distinctly depressed by the more concentrated antigen. The normal range of histone concentration is indicated by the bar at lower right of each graph.

The antigen-induced changes in the color of the A-S staining are accompanied by a change in the relation between wavelength and extinction, as shown in Fig. 3. This figure also demonstrates that the decrease in absorption at 480 mμ (E_{480}) is commonly associated with an elevation in the extinction value at 700 mμ (E_{700}). Thus, control thymocytes were characterized by E700 values which ranged from about 0.10 to 0.25, whereas the E_{700} values for the antigen-affected thymocytes ranged between 0.35 and 0.50. It therefore appears that the induced changes in A-S staining are associated with an increase in the black-staining fraction (E_{700}) as well as with a decrease in the stainability of the yellow-staining histone fraction as determined by subtracting E_{700} from E_{480}. It should be noted, however, that the decrease in the yellow-staining fraction appears to precede the increase in the black staining fraction. Table II documents the acute decrease in the yellow-staining fraction without a concomitant increase in the black-staining fraction.

TWICE CONCENTRATED TETANUS TOXOID: In contrast to the effects of the diluted antigen, the injection of twice concentrated tetanus toxoid produced only a slight decrease in the A-S–stainable

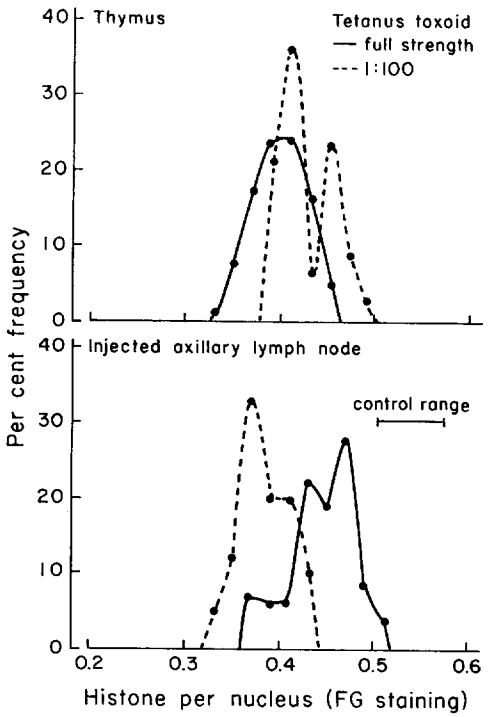

FIGURE 2 Relative amounts of fast green (FG)–stainable nuclear histones of thymocytes and regional lymph node lymphocytes 1 hour after the injection of full strength and diluted tetanus toxoid. Note that the diluted antigen produces a greater decrease in the lymphocyte histone than does the full strength antigen, confirming the changes depicted in Fig. 1.

nuclear histone within the 2-hour period after the injection. At the 5-hour period, however, the A-S staining was decreased and changed in color from a yellowish brown to blackish. Twenty-four hours after the injection, the control type of staining was almost completely regained. Thus the twice concentrated antigen produced a delay in the response as compared with the diluted antigen. After this delay, however, the antigen-induced changes were similar.

FULL STRENGTH TETANUS TOXOID: As indicated in Figs. 1 and 2, only minimal decreases in A-S and FG stainability of local lymphocyte nuclei followed the injection of full strength antigen within the first hour. Within 2 hours after the injection, however, the stainability fell to values which were similar to those obtained after the injection of the diluted antigen. That is, the results were intermediate between those following the concentrated and the diluted tetanus toxoid.

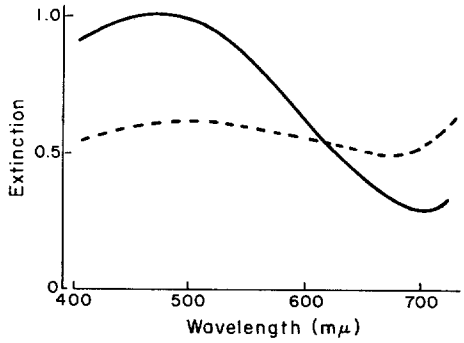

FIGURE 3 Relation between extinction and wavelength of ammoniacal-silver–stained axillary lymph node lymphocytes shown in Fig. 4. The solid line represents the yellowish lymphocytes from the cortisone-"protected" side; the dashed line is the curve of the black-staining antigen-affected lymphocytes.

TABLE II

Relative Intensity of Ammoniacal-Silver–Feulgen Staining of Control and Tetanus Toxoid–Affected Thymocytes

Group	(1) E_{440}/F	(2) E_{700}/F	(1) − (2)
Control	1.05	0.15	0.90
Tetanus toxoid 1:100			
30 minutes	0.85	0.11	0.79
1 hour	0.83	0.13	0.70
Tetanus toxoid full strength			
30 minutes	0.77	0.09	0.68
1 hour	0.81	0.27	0.54

Thymus

DILUTED TETANUS TOXOID: In contrast to its effect on the axillary lymph nodes, the injection of the dilute antigen produced a minimal decrease in the intensity of A-S and FG staining of the thymocyte nuclei and no change in the quality (yellowish brown) of A-S staining throughout the period of study. The differences between the responses of the thymocytes and the lymph node lymphocytes are recorded in Figs. 1 and 2. The staining of the thymocyte nuclei was greater than that of the lymphocyte nuclei, 1 and 2 hours after the injection of the diluted antigen.

CONCENTRATED TETANUS TOXOID: Within 30 minutes after the injection of the concentrated

antigen, the A-S stainability of the thymocyte nuclear histone decreased almost 30 per cent and the color of the staining became blackish. By 1 and 2 hours after the injection, the A-S stainability had continued to decrease while the FG stainability fell approximately 20 per cent below the control values. Such changes in the thymocytes following the injection of the concentrated antigen were in contrast to the minimal changes in the nuclei of the lymph node lymphocytes. The stainability returned toward the control values, however, by 5 to 24 hours.

FULL STRENGTH TETANUS TOXOID: The effects of the full strength dosage were similar to, although less marked than, those produced by the concentrated antigen. With both these dosages the intensity of staining of thymocyte histones 1 hour after the injection was lower than that of the lymph node lymphocytes, and remained depressed with little change at the 2-hour period. It will be noted that at the 2-hour mark the staining of the lymphocytes of the mice injected with the full strength antigen was similar to that of the thymocytes from the same mice. On the other hand, the intensity of staining of the thymocytes from mice injected with the doubly concentrated antigen was lower than that of the corresponding lymphocytes.

HETEROLOGOUS SERUM: One hour after the injection of 0.1 ml of human serum into the forelimb of a mouse there was a decrease in the A-S staining of nuclei of the lymphoid cells of both the thymus and the draining axillary lymph nodes. The qualitative and quantitative changes in the lymphocytes and thymocytes were similar to those occurring 2 hours after the injection of full strength tetanus toxoid. Similar effects were produced by the injection of 0.1 ml of a 16.5 per cent solution of human gamma globulin. Whereas a 2-fold increase in the tetanus toxoid caused a delay in the effect on the nuclear histones, no such delay was found after the administration of a similarly increased dosage of heterologous protein.

PRIOR INJECTION OF CORTISONE: The injection of 2.5 mg of cortisone into the upper forelimb, 15 minutes before the injection of full strength tetanus toxoid, was found to prevent the changes which the antigens caused in the histones of the lymphocytes of the axillary nodes. After the A-S and FG staining, the lymphocytes from such nodes appeared similar to control lymphocytes. In contrast, however, the lymph nodes injected with saline instead of cortisone, before the injection of tetanus toxoid, exhibited all the antigen-induced changes in their nuclear histones. Such lymphocyte nuclei revealed a decrease in the intensity of staining by A-S and FG, and showed a blackish color with A-S.

The differences in the effect of the prior injection of cortisone and saline on the antigen-induced changes in lymphocyte nuclear histones are documented in Fig. 4. This figure demonstrates that the intensity of staining of the histone of the saline-toxoid–treated lymphocyte nuclei was similar to that of lymphocyte nuclei after toxoid alone. In contrast, the A-S and FG staining of the cortisone-toxoid–treated lymphocytes was similar to that of control lymphocytes.

A-S–FEULGEN STAINING: Measurements of the AS-F–stained cells confirmed the changes demonstrated by measurements of the nuclei stained with A-S alone. As indicated in Table II, antigen injection was followed by a decrease in the intensity of E_{440}/F ratio. This decrease was due to a decrease in the yellow-stainable histone (E_{440} minus E_{700}). In contrast, the E_{700}/F ratio tended to increase.

COMMENTS

The present study has confirmed our previous observations that the injection of an antigen has the effect of inducing an acute reversible decrease in the A-S and FG stainability of nuclear histones of lymphoid cells of the thymus and lymph nodes. It has also been observed that the effect on the local lymph node lymphocytes is less acute after the administration of full strength and doubly concentrated tetanus toxoid than after 1:100 dilutions of tetanus toxoid. This delay is suggestive of the phenomenon of immunological paralysis, wherein a high dose of an antigen may produce tolerance rather than antibody production. In this connection it should be recalled that histones tend to form precipitates with acid proteins. However, such precipitates usually dissolve in the presence of excess histone. It would be of interest to know whether a similar situation obtains in the reaction between antigens and the histones of lymphoid cells. That is, does antigen excess lead to the formation of insoluble antigen-histone complexes with resultant immunological incompetence? The lack of such a "paralysis" effect on the thymocyte histones might reflect intrinsic differences between thymocytes and lymphocytes in regard to their response to antigens. Such differences are sug-

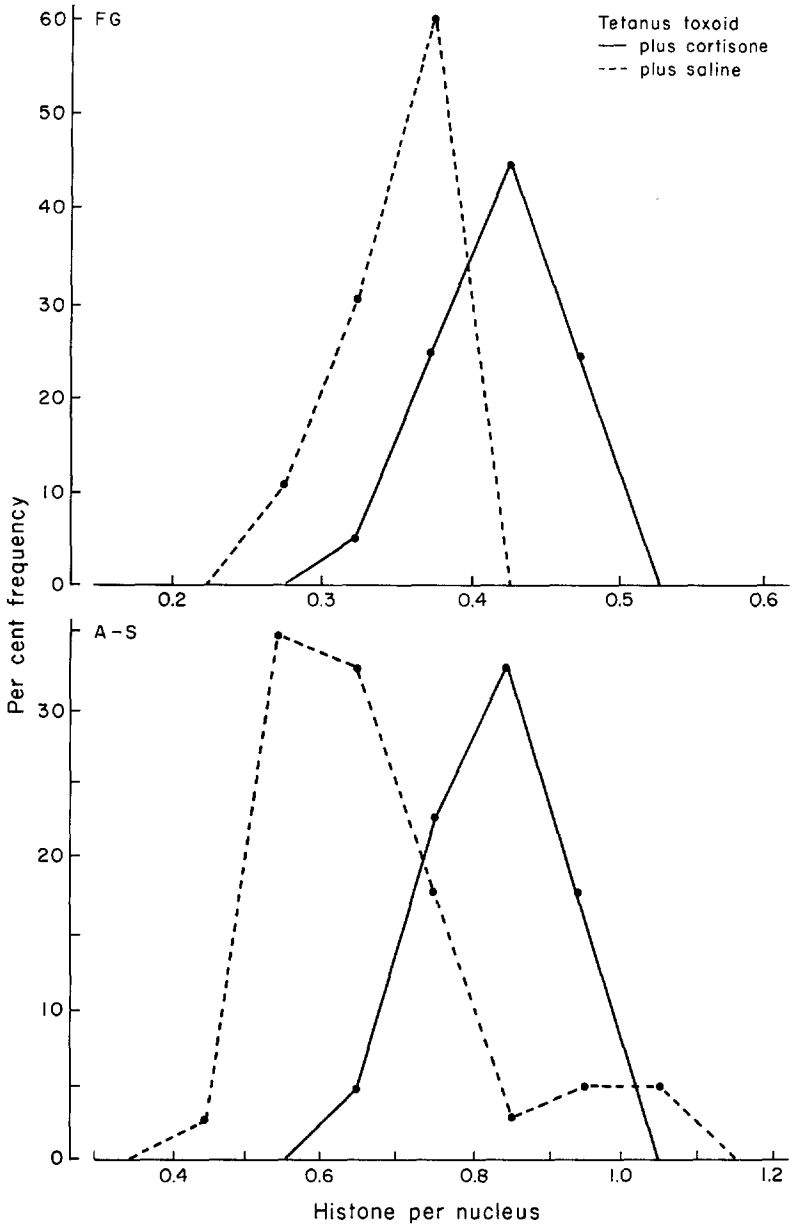

FIGURE 4 Fast green (FG) and ammoniacal-silver (A-S) staining of histones of lymphocytes from right and left axillary lymph nodes of the same mouse 2 hours after the injection of cortisone and tetanus toxoid into the left forelimb and saline and tetanus toxoid into the right forelimb. Note that the side exposed to saline and toxoid shows the usual antigen-induced decrease in FG- and A-S-stainable histone. No such change occurs on the cortisone-"protected" side.

gested by the fact that the color of A-S–stained axillary node lymphocyte nuclei tends to be even more yellowish than that of thymocyte nuclei, thus indicating a somewhat different histone composition. Nevertheless, the probability that the difference is a function only of the concentration of the antigen reaching the cells is supported by other studies wherein we observed that the injec-

tion of concentrated tetanus toxoid into a hind foot pad of a guinea pig caused an acute decrease in the A-S staining of the inguinal lymph node lymphocytes, while the lymphocytes of the popliteal nodes were unchanged. It would seem that further investigations are needed in order to define the detailed relation between antigen concentration and the nuclear histones of different types of lymphoid cells.

It is well known that cortisone may depress immunological competence and exert a lymphocytolytic effect. It was therefore of interest to find that cortisone inhibited the antigen-induced decrease in histones in lymphoid cells. Such findings would suggest, though it remains to be proved, that the antigen-induced changes in the nuclear histones of lymphoid cells are related to immunological competence.

The data do not permit a definitive explanation of the biochemical basis of the antigen-induced changes in the FG and A-S staining. The decrease in stainability might represent an actual loss of histones from the nuclei of lymphoid cells. Such a liberation of highly reactive molecules might bind antigens in a non-specific fashion or serve as instructional material if taken up by reticular cells. On the other hand, the data might also be explained by a change in the reactive groups of the histones without any discharge of nuclear histone. It will be recalled that the FG staining and particularly the A-S staining depend on the presence of free ϵ-amino groups of lysine. Blocking of such groups by acetylation, deamination, or combination with non-histone proteins (antigen ?) could decrease the stainability of such altered histones. It should be mentioned that the prior treatment of lymphoid cells with nitrous acid (HNO_2) causes a loss of subsequent yellow A-S staining with an accompanying decrease in the E_{480} value but without any appreciable effect on the E_{700} value.

Similar observations have been made in regard to the A-S staining of isolated thymus histones on cellulose acetate strips; that is, the "loss" of the yellow-staining histone is not necessarily associated with a concomitant transformation to black-staining histone. Measurements of dry mass of the nuclei by means of interferometry might prove rewarding in determining whether the observed changes involve an actual loss of protein from the nucleus.

It should be noted, however, that either the loss of histones or the neutralization of histone amino groups may alter the RNA synthetic function of DNA (7). It is interesting to speculate on the question whether the observed changes in histones play a role in the transformation of lymphoid cells into antibody-producing cells or whether RNA produced by lymphocytes may be transferred to reticular cells which in turn acquire the ability to produce antibody and are transformed into plasma cells. Though the available data are incomplete, they suggest that the antigen-induced changes in the lymphoid cells play a significant and a hitherto unsuspected role in the cellular and humoral response to antigen.

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