DIFFERENTIATION AND FUNCTIONAL EXPRESSION OF POTENTIAL ANTIBODY-PRODUCING CELLS IN THE PRESENCE OF CHLORAMPHENICOL

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ABSTRACT

Rabbits were immunized with diphtheria toxoid combined with complete Freund's adjuvant. Half of the animals were started on intramuscular injections of chloramphenicol 24 hr before the injection of the antigens. There was a general depression of protein synthesis in the immune system in the presence of chloramphenicol, but a greater effect on the synthesis of antibody than on the synthesis of proteins necessary for reproduction and maturation. In contrast to the finding of antibody in cells of the spleen and in the circulation of the control animals, those animals receiving chloramphenicol did not have measurable circulating antibody, and their spleens contained only a few cells with intracytoplasmic antibody late in the course of the experiment. Cytologically there was maturation of potential antibody-producing cells in the red pulp and nonfollicular white pulp of the spleen while the animals were receiving chloramphenicol. These cells developed more slowly, and were fewer and smaller than those of the control animals. They had numerous small, electron-opaque particles in their cytoplasm early in development. Ribosomes were synthesized, though fewer in number. The endoplasmic reticulum formed more slowly.

INTRODUCTION

In bacteria and bacterial cell-free systems chloramphenicol (CM) has been shown to have an inhibitory effect on protein synthesis. In contrast to this, mammalian systems are relatively insensitive to the antibiotic. The notable exception seems to be antibody synthesis.

When CM is administered in sufficient quantity before immunization, or early in the inductive phase of antibody synthesis, there is an attenuation of the formation of antibody. This has been shown in animals (1–5) and in cultures of lymphoid tissue from immunized animals (6–8). Those observations were concerned with the effects of CM on circulating antibody and synthesis of antibody in cultures of lymphoid tissue. There is little information about the cellular aspects of the immune process in the presence of CM.

In this study the parameters considered were the effect of CM on: (*a*) antibody synthesis in terms of circulating antibody and intracellular antibody; (*b*) the maturation of immunologically competent cells, particularly the development, in the walls of the sinusoids of the red pulp of the spleen, of mononuclear cells associated with the synthesis of γM antibody, and the development, in the nonfollicular white pulp of the spleen, of plasma cells associated with γG antibody synthesis; and (*c*) mitotic activity in these areas.

MATERIALS AND METHODS

Albino rabbits of both sexes weighing between 1.5 and 2.0 kg were maintained on Purina Chow and tap water.

Chloramphenicol (CM)

Chloramphenicol (Parke-Davis and Co., Ann Arbor, Michigan) was prepared as a suspension with carboxymethyl cellulose in saline (1 g of CM, 10 mg of carboxymethyl cellulose in 3 ml of 0.15 M NaCl). Intramuscular injections of this suspension containing 0.6 g of CM/kg of body weight, into the hind flanks of the animals, resulted in sustained blood levels and uniformly attenuated antibody synthesis with the antigens used. CM was started 1 day before immunization and continued to the end of the experiment. Half the daily dose was given at approximately 12-hr intervals. Serum levels of CM were determined by the method of Glazko, Wolfe, and Dill (9), averaging 10 μ/ml (8–12 μ/ml).

Antigens

Soluble diphtheria toxoid (390 Lf/ml) was obtained from the Lederle Division, American Cyanamid Co., Pearl River, New York, and complete Freund's adjuvant containing 8.5 ml Bayol F (Liquid Petrolatum, U.S.P.), 1.5 ml of Arlacel and heat-killed, desiccated *Mycobacterium butyricum* 5 mg/10 ml, from Difco Products Co., Chicago, Ill.

A soluble antigenic fraction from *Mycobacterium butyricum* was prepared by extracting 100 mg of heat-killed, dried *Mycobacterium butyricum* (Difco) with 10 ml of Tris buffer, pH 7.0 and ionic strength 0.15. The suspension was kept at 4°C for 1 wk with intermittent stirring. It was centrifuged and the supernatant used for the immunofluorescence studies. The soluble fraction formed specific immune precipitates and fixed complement in the presence of specific antisera.

Antisera

Antisera to diphtheria toxoid were prepared by repeated intravenous injection of rabbits with soluble diptheria toxoid without Freund's adjuvant.

Antisera to *Mycobacterium butyricum* were obtained by immunizing rabbits with 1 ml of the complete adjuvant and bleeding the animals between 21 and 28 days after inoculation. Only one injection was necessary; repeated injections did not yield elevated titers (10).

Sheep anti-rabbit γ-globulin was obtained from Arnel Products Co., New York, N. Y.

Antibody Assays

Antibody titers to *Mycobacterium butyricum* were measured by complement fixation (10), and titers to diphtheria toxoid by hemagglutination (11).

Electrophoresis

The distribution and level of the serum proteins were determined on sera from all animals by paper electrophoresis using Veronal buffer, pH 8.6, ionic strength 0.05. Staining for protein was done by the bromphenol blue procedure. During the time the animals were receiving CM, the distribution and level of serum proteins were the same as in the control animals.

Immunofluorescent Procedures

Intracellular γ-globulin and γM antibody and γG antibody to diphtheria toxoid and *Mycobacterium butyricum* were identified by immunofluorescence.

Globulin fractions were prepared by precipitation from the immune sera at 50% saturation with $(NH_4)_2SO_4$. The precipitates were dissolved in small volumes of phosphate saline buffer, pH 7.0, ionic strength 0.15, and dialyzed for 24 hr against a large volume of the same buffer at 4°C.

Aliquots of each of the antibody-containing globulin fractions were conjugated with fluorescein isothiocyanate, dialyzed exhaustively against phosphate saline buffer, pH 7.0, ionic strength 0.15, absorbed with activated charcoal and centrifuged before use. Prior to and after conjugation, each was checked for antibody titer, specific antibody, and specificity of staining.

Portions of the spleen from each of the animals were fixed in 95% ethanol at 4°C for 24 hr and processed according to the method described by Sainte-Marie (12). Intracellular γ-globulin was identified by the direct immunofluorescence method, using the globulin fraction of sheep anti-rabbit γ-globulin conjugated with fluorescein isothiocyanate. The indirect method was used to identify specific antibody to the antigens.

To identify γM antibody in the cells, sections of spleen were incubated with goat anti-rabbit γM globulin followed by soluble diphtheria toxoid or soluble *Mycobacterium butyricum* antigen and then the labeled rabbit anti-toxoid or anti-*Mycobacterium butyricum* globulin. The absence of fluorescence when compared with the controls indicates the presence of γM antibody. γG antibody was identified by treating adjacent sections with goat anti-rabbit γG globulin followed by soluble diphtheria toxoid or soluble *Mycobacterium butyricum* antigen and then the labeled anti-toxoid or anti-*Mycobacterium butyricum* antibody.

The goat anti-rabbit γ M antibody was prepared as described previously (13). The goat anti-rabbit γG antibody was generously given by Dr. A. Feinstein, Cambridge University.

In all instances, fluorescent staining could be blocked or markedly attenuated by treatment of the sections with the appropriate unlabeled fractions before the conjugated antibody.

Histologic Preparations

Samples of spleen used for histologic examination were fixed in formaldehyde, embedded in paraffin, and sections were stained with hematoxylin and eosin. As a gross estimate of RNA, adjacent sections were stained with toluidine blue, with and without treatment of the sections with RNase.

Electron Microscope Preparations

Samples of spleen were fixed in phosphate-buffered osmium tetroxide (pH 7.2 to 7.4) containing sucrose and embedded in Maraglas. Sections of approximately 500 A were stained with uranyl acetate and lead hydroxide and examined in an RCA-2D electron microscope.

EXPERIMENTAL

The rabbits were separated into two groups and immunized by injection into the marginal ear vein.

Group A These rabbits received 1 ml of complete Freund's adjuvant, followed immediately by 80 Lf of soluble diphtheria toxoid through the same needle. The spleens were removed at 3, 6, 7, 9, 11, 13, 15, 17, and 21 days after immunization.

Group B A similar series of animals was started on 0.6 g of CM/kg of body weight the day before immunization and continued to the end of the experiment.

The rabbits were bled at intervals from the marginal ear vein. The sera were not pooled. At the indicated times, four animals in each group were anesthetized with sodium pentobarbital; blood was obtained from the heart, and the spleens were removed for histologic, immunofluorescent, and electron microscope examination (10, 13–15).

Complete Freund's adjuvant was used along with diptheria toxoid in order to place a greater stress on the immune system. The addition of the adjuvant increases the proliferation of cells associated with antibody production and results in an accelerated, enhanced, and prolonged synthesis of both γM antibody and γG antibody (13, 14). In addition, the adjuvant causes a temporal separation of the γM and γG responses so that they can, to some extent, be studied separately.

RESULTS

The general pattern of the immune response in rabbits treated with complete Freund's adjuvant and diphtheria toxoid has been described previously (10, 13–15). Circulating antitoxoid and antibody to *Mycobacterium butyricum* were not found, by the methods used, in the sera from those animals that were maintained on CM (Tables I and II). This was in contrast with the relatively high levels of circulating antibody obtained in those animals not receiving the antibiotic.

Development of Cells Associated with Antibody Synthesis

The cells that are eventually concerned with the synthesis of γG antibody and γM antibody are limited to lymphocytic cells of the nonfollicular white pulp and nonphagocytic mononuclear cells (large lymphocyte-like) in the walls of the sinusoids of the red pulp, respectively. They can be shown to contain specific antibody several days before that particular species of antibody can be demonstrated in the circulation by the methods employed. It should be emphasized that the absence of demonstrable antibody in the circulation does not represent complete inhibition of antibody synthesis. In these studies it is necessary to consider intracellular as well as circulating antibody.

Mononuclear Cells

3 days after immunization with adjuvant and toxoid certain nonphagocytic mononuclear cells in the walls of the sinusoids of the red pulp showed an increased amount of basophilic cytoplasm that was RNase sensitive. Associated with this was a substantial increment in mitotic activity that correlated with the larger number of these cells that were eventually found (Fig. 1). From 6 days onward, these cells contained γ-globulin and specific γM antibody. The number of cells containing γM antitoxoid steadily increased until 13 days had elapsed, and then declined sharply, so that after this time antitoxoid was rarely found in these cells. In contrast, γM antibody to *Mycobacterium butyricum* was present in fewer cells of this type during the first 13 days, but the number with this antibody continued to increase throughout the experiment, being more numerous at 17 and 21 days (Tables I and II).

In those animals that were given CM, there was a delay in the development of the mononuclear cells in the walls of the sinusoids of the red pulp. Though the amount of cytoplasm increased, it did so more slowly than in the controls. RNase-sensitive basophilia of the cytoplasm was not appreciated until 11 or 12 days after immunization. Mitotic figures were not so frequent in this group, but were more numerous than in nonimmunized animals or in animals that received only toxoid (10). There were no abnormal mitoses. Cells of this kind were never so plentiful as in the animals

TABLE I

Antibody and Cellular Response to Diphtheria Toxoid Given with Complete Adjuvant with and without Chloramphenicol (CM)

Days:		3	6	7	9	11	13	15	17	21
Circulating antitoxoid*	no CM:	0	20	40	640	1280	2560	2560	2560	2560
	CM:	0	0	0	0	0	0	0	0‡	0
Species of antibody in circulation of control animals	% γM:	0	§	100	100	100	80	80	70	70
	% γG:	0	§	§	§	§	20	20	30	30
Development mononuclear cells‖	no CM:	few	+	+	2+	2+	3+	3+	3+	4+
	CM:	0	0	0	0	few	few	+	2+	3+
Estimated % mononuclear cells with antitoxoid¶	no CM:	0	75	75	75	75	75	25	5	5
	CM:	0	0	0	0	0	0	0	5	5
Development mature plasma cells‖	no CM:	0	0	0	few	+	2+	3+	3+	4+
	CM:	0	0	0	0	few	+	2+	2+	2+
Estimated % mature plasma cells with antitoxoid¶	no CM:	0	0	0	50	50	50	50	50	50
	CM:	0	0	0	0	0	0	0	0	5

* Reciprocal of titers of circulating antibody.

‡ One animal had a titer of 1/160 at this time.

§ Insufficient antibody titers for determination of species of antibody.

‖ These represent the total number of cells of these types.

¶ The percentages represent the cells containing antitoxoid.

TABLE II

Antibody and Cellular Response to Mycobacterium Butyricum in Freund's Adjuvant Given with Diphtheria Toxoid with and without Chloramphenicol (CM)

Days:		3	6	7	9	11	13	15	17	21
Circulating antibody to mycobacteria*	no CM:	0	0	2–4	12	24	32	64	128	192
	CM:	0	0	0	0	0	0	0	0	0
Species of antibody in circulation of control rabbits	% γM:	0	0	‡	‡	‡	100	100	100	100
	% γG:	0	0	‡	‡	‡	‡	‡	‡	trace
Development mononuclear cells§	no CM:	few	+	+	2+	2+	3+	3+	3+	4+
	CM:	0	0	0	0	few	few	+	2+	3+
Estimated % mononuclear cells with antibody to mycobacteria‖	no CM:	0	25	25	25	25	25	75	95	95
	CM:	0	0	0	0	0	0	0	0	5
Development mature plasma cells§	no CM:	0	0	0	few	+	2+	3+	3+	4+
	CM:	0	0	0	0	few	+	2+	2+	2+
Estimated % mature plasma cells with antibody to mycobacteria‖	no CM:	0	0	0	50	50	50	50	50	50
	CM:	0	0	0	0	0	0	0	0	5

* Reciprocal of titers of circulating antibody.

‡ Insufficient antibody titers for determination of species of antibody.

§ These represent the total number of cells of these types.

‖ The percentages represent the cells containing antibody to *Mycobacterium butyricum*.

that did not receive CM but were substantially greater than seen in animals not receiving adjuvant (Fig. 2). Antitoxoid and antibody to *Mycobacterium butyricum* were found in very few of these basophilic mononuclear cells, and then only 17 to 21 days after immunization (Tables I and II). The delay and slower rate of maturation was substantial, indicating a suppressive effect of CM on structural protein synthesis as well as antibody synthesis.

Plasma Cells

The spleens from the animals that were immunized but not treated with CM had plasma cells, containing γG antibody, in and adjacent to the nonfollicular white pulp, and appeared to develop from the small and medium lymphocytic cells in this area (14, 15). Mitotic activity was greater in these regions compared with similar areas in the animals that received CM. 7 days after immunization, some of the small and medium-sized lymphocytes enlarged and accumulated an abundant RNase-sensitive basophilic cytoplasm. At this stage these cells resembled developing plasma cells (Fig. 3). There were a few classical plasma cells 9 days after immunization, increasing in number until 21 days had elapsed. (Fig. 4). Beginning at 13 days, there was a gradual reduction in the cytoplasmic basophilia of the plasma cells and a transition toward acidophilia. γ-globulin, γG antitoxoid, and γG antibody to *Mycobacterium butyricum* were found in plasma cells by 9 days and thereafter in an an increasing number of these cells (Tables I and II).

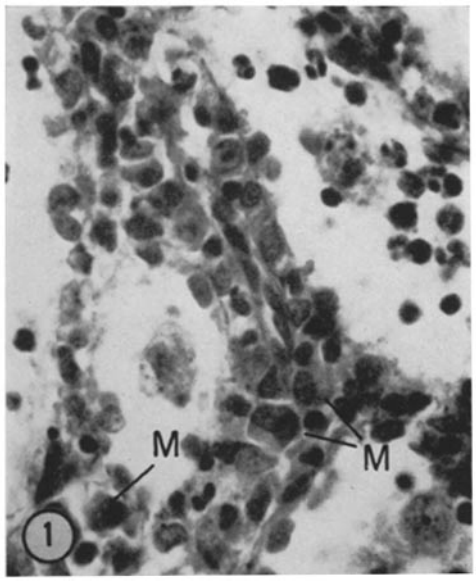

FIGURE 1 Mononuclear cells (*M*) in the walls of the sinusoids of the red pulp of the spleen 11 days after immunization with complete Freund's adjuvant and diphtheria toxoid. These cells have a moderate amount of RNase-sensitive basophilic cytoplasm and are of the kind that contain γM antibody and γ-globulin. Hematoxylin and eosin. × 640.

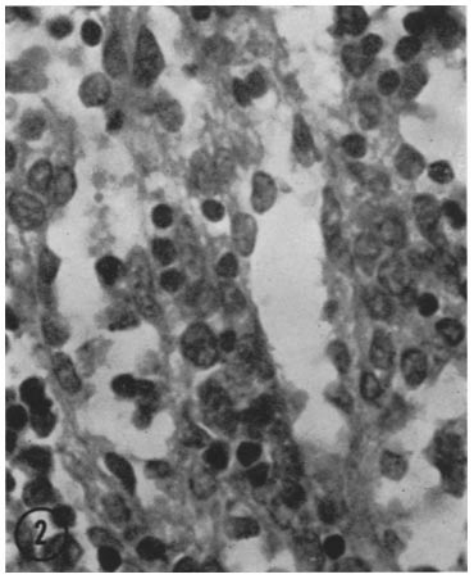

FIGURE 2 Area of the red pulp, 11 days after immunization, from an animal that was given chloramphenicol in addition to complete Freund's adjuvant and diphtheria toxoid. The mononuclear cells are not so common and their cytoplasm is less abundant, compared with the animals that did not receive the drug. Specific antibody and γ-globulin was rarely found in the cytoplasm of these cells. Compare with Fig. 1. Hematoxylin and eosin. × 640.

The delay in the maturation of plasma cells was similar to that of the mononuclear cells in the animals receiving CM. A few lymphocytic cells in the nonfollicular white pulp had an increase of basophilic cytoplasm by 7 days (Fig. 5). Mitotic activity was less than in the untreated group. Mature plasma cells were not found until 11 days. After this time, the number of mature plasma cells increased, but they were never so plentiful as in the animals that did not receive the drug (Fig. 6) (Tables I and II). Antitoxoid and antibody to *Mycobacterium butyricum* were not found in the plasma cells until 21 days after injection of the antigens, and then in only very few of the cells.

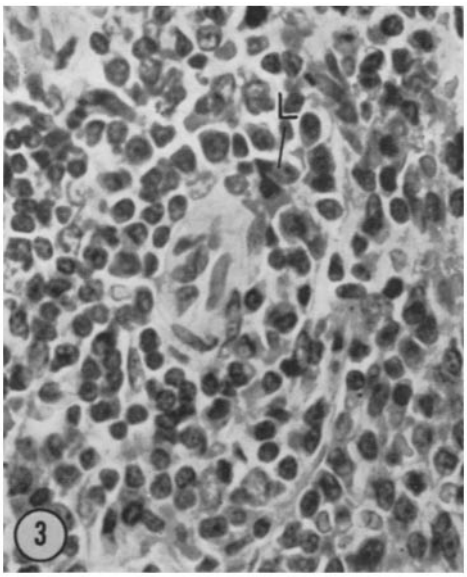

FIGURE 3 Area of the nonfollicular white pulp 7 days after immunization with complete adjuvant and diphtheria toxoid. There are a number of lymphocytic cells with increased amounts of RNase-sensitive basophilic cytoplasm. A cluster of these cells is shown at *L*. Hematoxylin and eosin. × 640.

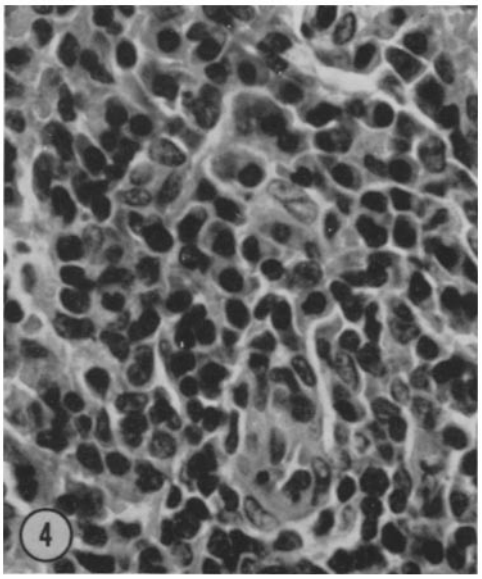

FIGURE 4 Numerous plasma cells in the nonfollicular white pulp of the spleen 21 days after immunization with complete Freund's adjuvant and diphtheria toxoid. These cells contain γG antibody and γ-globulin in their cytoplasm. Hematoxylin and eosin. × 640.

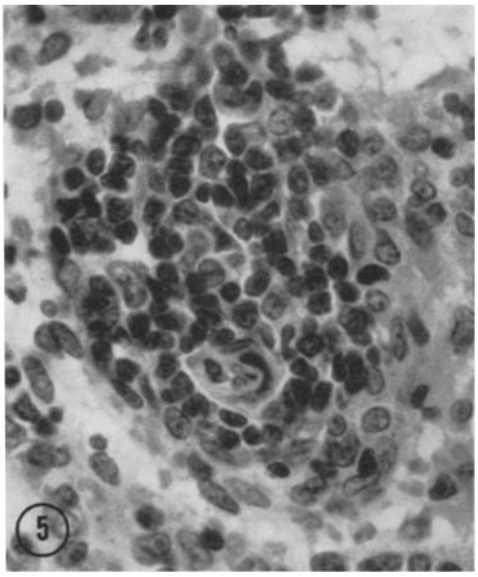

FIGURE 5 The nonfollicular white pulp, 7 days after immunization with complete adjuvant and diphtheria toxoid, in an animal treated with chloramphenicol. The lymphocytic cells show little increase in basophilic cytoplasm at this time, compared with those of animals that were not given chloramphenicol. Compare with Fig. 3. Hematoxylin and eosin. × 640.

The total number of cells containing antitoxoid and antibody to *Mycobacterium butyricum* in all animals was generally comparable to the number containing γ-globulin. With respect to time, staining for γ-globulin was similar to that for specific antibody. However, in terms of cellular maturation and its relationship to antibody synthesis, the number of mononuclear cells and plasma cells showing cytoplasmic basophilia in the animals receiving CM was always greater than the number containing γ-globulin or antibody. In contrast, the cytoplasmic basophilia and maturation of the antibody-producing cells in the control animals were directly paralleled by their content of antibody and γ-globulin.

Electron Microscopy

By combining electron microscopy with conventional methods, it was possible to trace the sequence of development of the immunologically competent cells in both the red and white pulps. Within 3 days after immunization without CM, the mononuclear cells of the red pulp had many ribosomes (Fig. 7). They were both randomly scattered and arranged in aggregates throughout

the cytoplasm (Fig. 8). A prominent Golgi complex and segments of endoplasmic reticulum were seen by 7 days. With the development of the endoplasmic reticulum, some of the ribosomes were attached to the lamellae. It was at this stage that the RNase-sensitive basophilia of the cytoplasm was most intense and γ-globulin and γM antibody could first be demonstrated. Once the mononuclear cells reached this level of development there was no further change. They did not achieve the ultrastructural details of the plasma cell.

Beginning at 3 days, some of the small- and medium-sized lymphocytes of the nonfollicular white pulp gradually developed endoplasmic reticulum, a complicated Golgi apparatus, and an increment of ribosomes. The ribosomes were free, in aggregates, and attached to the lamellae of the endoplasmic reticulum by 6 days (Figs. 7 and 8). 13 days after immunization, these cells had the currently accepted ultrastructural characteristics of mature plasma cells. These changes paralleled the increase in RNase-sensitive basophilic cytoplasm and the appearance of γ-globulin and γG antibody. As the number of plasma cells containing antibody and the intensity of fluorescence increased and the cytoplasmic basophilia decreased, electron-opaque, amorphous material accumulated in the channels between the lamellae of the endoplasmic reticulum.

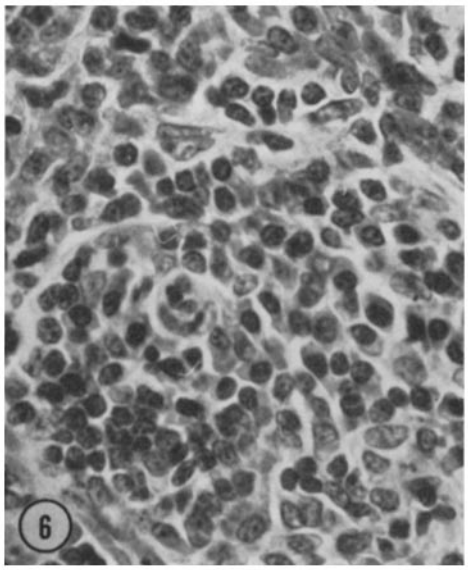

FIGURE 6 An area from the nonfollicular white pulp, 21 days after immunization with complete Freund's adjuvant and diphtheria toxoid, in an animal that received chloramphenicol. There are a number of lymphocytic cells with increased amounts of basophilic cytoplasm but fewer mature plasma cells than seen in animals that did not receive chloramphenicol. Only a few of the plasma cells contained demonstrable specific antibody and γ-globulin. Compare with Fig. 4. Hematoxylin and eosin. × 640.

In the animals receiving CM in addition to the adjuvant and toxoid, there was a delay in the maturation of the cytoplasm of 2–3 days in both the mononuclear and plasma cells. During the period of development, the cells differed in several ways from those of the control group. There was a smaller increment of cytoplasm. Ribosomal particles were common, but aggregation not so extensive. In addition, the cytoplasm was loaded with numerous, small electron-opaque particles (Figs.9 and 10). This marked accumulation of small electron-opaque particles in the cytoplasm is not found in the usual sequence of development of the antibody-producing cells in animals not receiving the antibiotic. There were fewer profiles of endoplasmic reticulum during this period and a corresponding delay in the development of the Golgi apparatus. By 17 days and after, mononuclear and plasma cells were morphologically similar to those of the control group (Fig. 11), and some of the plasma cells contained electron-opaque, amorphous material between the lamellae of the endoplasmic reticulum.

DISCUSSION

The effect of CM on the attenuation of antibody synthesis can be considered in relation to the sequence of events thought to be necessary for a primary immunologic response, e.g. phagocytosis; relay of a stimulus to potential antibody-producing cells; and subsequent proliferation, maturation, and functional expression of the recipient cells.

It is apparent that CM interfered significantly with the synthesis of specific antibody (i.e. γM antibody and γG antibody) and to a somewhat lesser extent with structural protein synthesis (i.e. proteins necessary for cellular reproduction and maturation).

There is no significant impairment of phagocytosis of antigen by splenic macrophages in animals receiving CM.[1] That some stimulus was received by potential antibody-producing cells in the presence of CM is illustrated by the increased

[1] R. D. Moore and M. D. Schoenberg, unpublished observations.

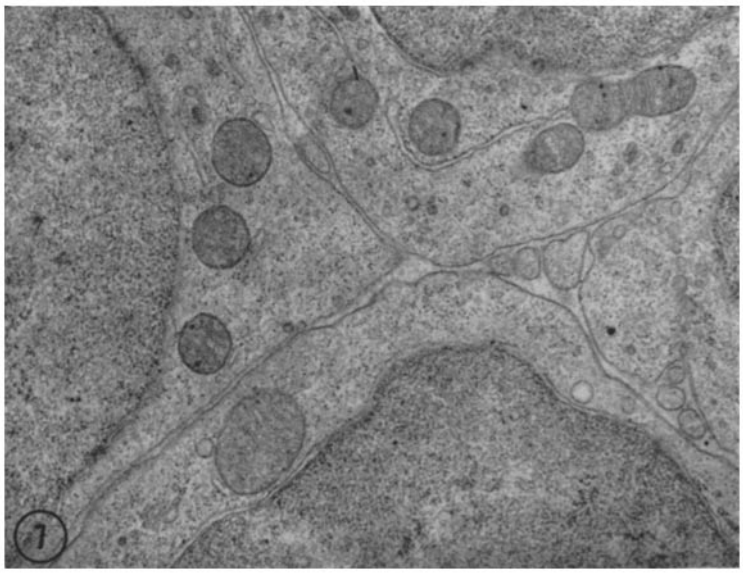

FIGURE 7 Electron micrograph illustrating ribosomes and a few segments of endoplasmic reticulum in the cytoplasm of developing immunologically competent cells of the nonfollicular white pulp. The illustration is representative of an animal 6 days after receiving complete Freund's adjuvant and diphtheria toxoid. Similar changes occur in the mononuclear cells of the red pulp. Ribosomes and endoplasmic reticulum progressively increase in amount in these cells, paralleling the RNase-sensitive basophilia of the cytoplasm. Uranyl acetate and lead hydroxide. × 19,000.

mitotic activity in these cells compared with that of cells of nonimmunized animals; the development of ribosomes, endoplasmic reticulum, and Golgi apparatus in these cells; and eventual antibody production by some. The rapid immune response following cessation of the antibiotic provides further evidence that immunocompetent cells have been prepared (5). Additional support comes from the fact that animals receiving CM failed to develop circulating antibody during the primary response, but had a characteristic secondary reaction when reexposed to the antigen (2).

There is a general depression of protein synthesis in the immune system in those animals given CM. This is manifest by a marked suppression of antibody production and by a delay and alteration in the maturation of the potential antibody-producing cells. The difference in sensitivity between the attenuation of antibody synthesis and the attenuation of structural protein synthesis is probably quantitative rather than qualitative.

Though mitotic activity was not quantitatively determined, mitoses were delayed and were fewer in the animals that received CM. However, mitotic activity was greater in the group receiving the drug than in nonimmunized animals or in animals given toxoid without adjuvant. A sufficient number of cells proliferated, so that antibody synthesis would have been expected if the number of immunologically competent cells available was the only consideration. In addition, the drug did not cause bizarre mitotic forms or impair the eventual return of these cells to normal function when the antibiotic was withdrawn (5).

Despite the delay in appreciation of the maturation of immunologically competent cells, a substantial number of ribosomes was formed in the presence of the drug. These ribosomes were similar in size and appearance to those found in the control animals within the limits of measurement (electron microscope examination of thin sections). In addition, early in the course of maturation (CM group) there were many smaller electron-opaque particles in the cytoplasm of these cells. The appearance of these smaller particles preceded and then paralleled the RNase-sensitive basophilia of the cytoplasm. This is similar to the enhancement of synthesis or accumulation of smaller, sedimenting RNP components described in bacterial systems in the presence of CM (16–18). Some of the

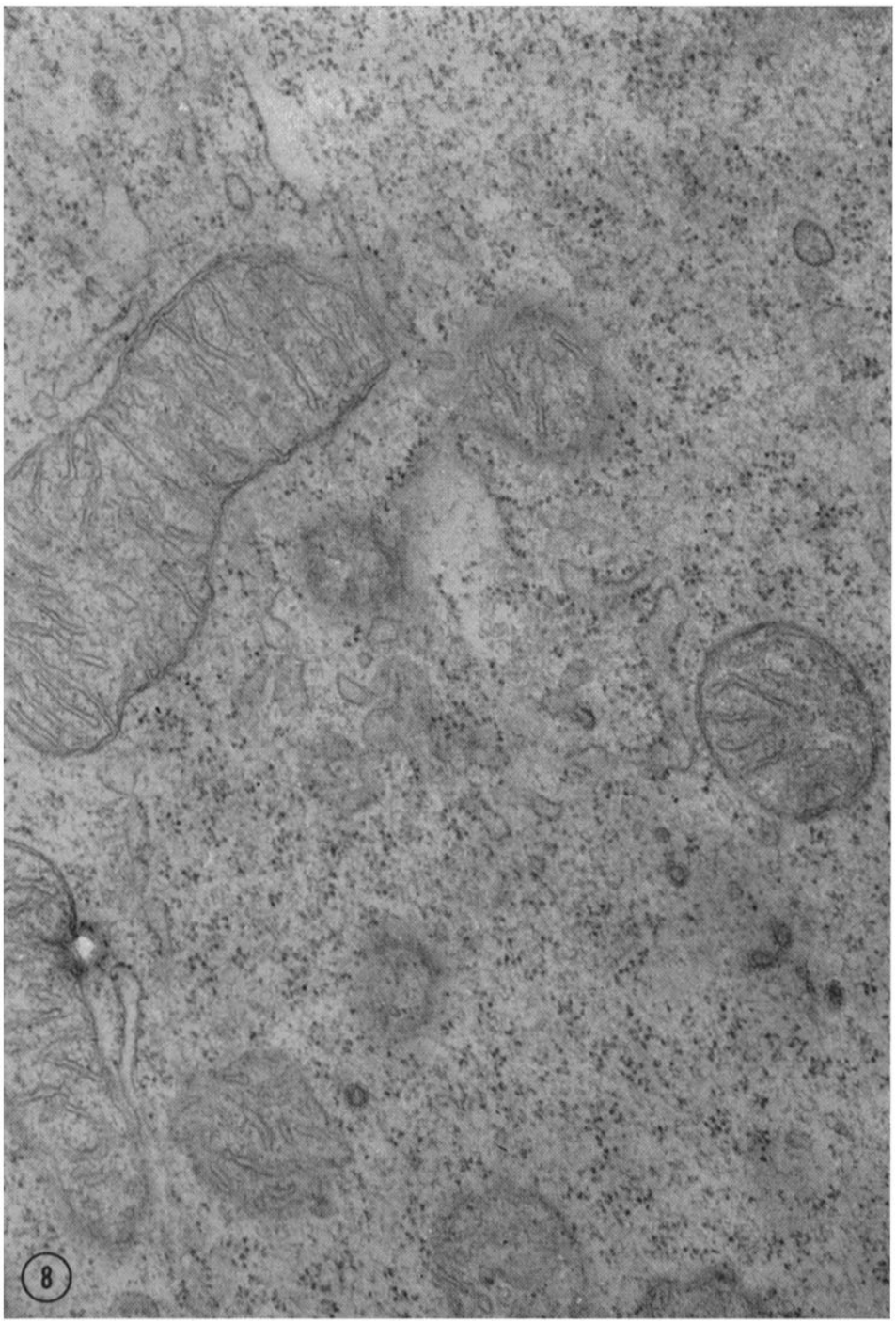

FIGURE 8 Electron micrograph representative of the random scattering and aggregation of RNP particles in the cytoplasm of the mononuclear cells in the red pulp and lymphocytic cells in the nonfollicular white pulp 6 days after immunization with complete Freund's adjuvant and diphtheria toxoid. RNP particles are also associated with the lamellae of the segments of endoplasmic reticulum. Uranyl acetate and lead hydroxide. × 50,000.

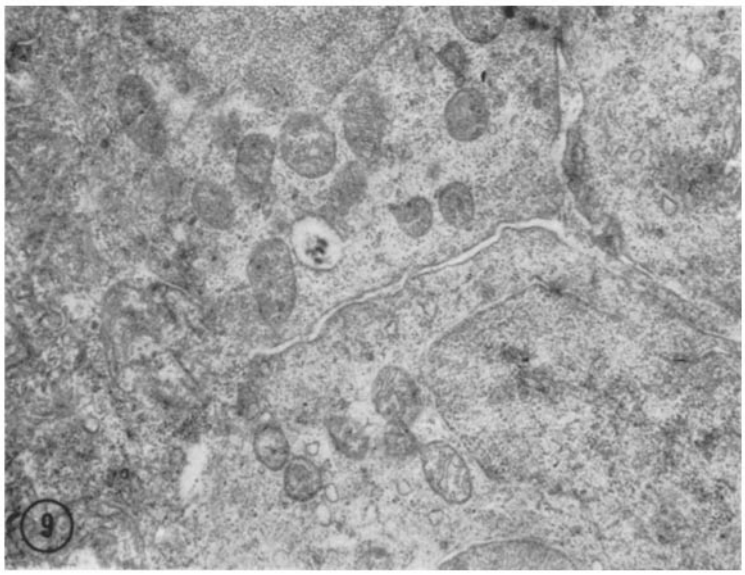

FIGURE 9 Electron micrograph of an area of nonfollicular white pulp representative of an animal receiving chloramphenicol and immunized 6 days previously with complete Freund's adjuvant and diphtheria toxoid. These cells are usually smaller, with a more electron-opaque cytoplasm; ribosomes are present and smaller electron-opaque particles are common. Uranyl acetate and lead hydroxide. × 19,000.

smaller particles probably represent the synthesis of ribosomal-RNA and/or protein-poor ribosomes (19, 20). Since CM is not known to attenuate RNA synthesis, the latter must be seriously considered.

Mitchell (21, 22) and Mach and Vassalli (23) reported that the injection of antigen results in an increase in RNA synthesis in the spleen and lymph nodes. They found that following immunization the initial rapid synthesis of RNA is mostly ribosomal rather than messenger RNA. This was particularly well illustrated by the rapid accumulation of labeled RNA in the microsomal fraction which, under the conditions of the experiments, contained the main part of the ribosomes.

If both mRNA and rRNA syntheses are maintained in the immunologically competent cells in the presence of the antibiotic and the prime effect of the drug is on protein synthesis, then the assembly of the synthetic machine (mRNA, rRNA- protein), the functional ribosome or polyribosome, might be impaired (19, 20, 24, 25). Quantitatively, this would express itself to a greater extent in antibody synthesis than in structural protein synthesis.

It is fairly well established that CM does not significantly affect protein synthesis by endogenous mRNA associated with mammalian ribosomes. This has been shown with rat liver ribosomes (26), rat liver microsomes (27) and rabbit reticulocyte ribosomes (28–30). The expression of some synthesis of structural proteins in the immunologic cells may be similar. Any endogenous mRNA-ribosome complex already formed would not be materially affected by CM. The delayed and decreased morphologic response could be accounted for by the necessity of manufacturing additional new protein-synthesizing systems directed to reproduction and structural development. CM apparently interferes with these processes.

With respect to antibody synthesis an entirely new message and synthetic machine must be constructed. That mRNA for antibody synthesis is delayed compared to rRNA would make the assembly of the polyribosome necessary for the formation of antibody more difficult. Though high doses of CM were maintained, a small percentage of cells eventually contained demonstrable antibody. This is not surprising since the synthesis of some normal ribosomes and endoplasmic reticulum takes place in the presence of the drug. An analogous situation has been found in the effect of CM on protein synthesis in chick fibroblasts (31). When these cells were making protein at a maximal rate, CM was without effect, but in cells just initiating protein synthesis, the drug sharply re-

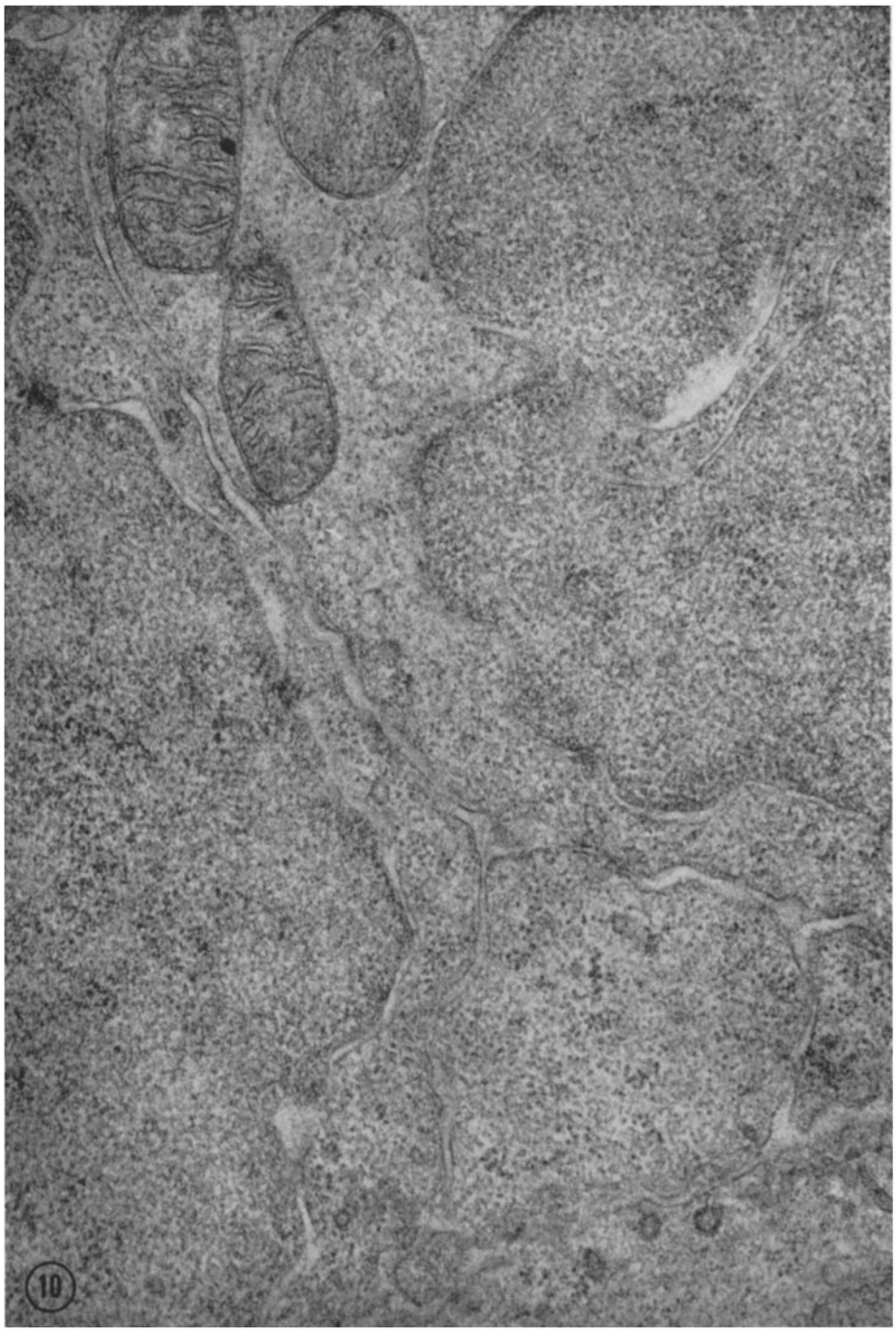

FIGURE 10 Electron micrograph illustrating the random scattering and aggregation of RNP particles in the animals receiving chloramphenicol. Complete adjuvant and diphtheria toxoid had been given 6 days before. Note the numerous smaller particles present throughout the cytoplasm. Compare with Fig. 8. Uranyl acetate and lead hydroxide. × 50,000.

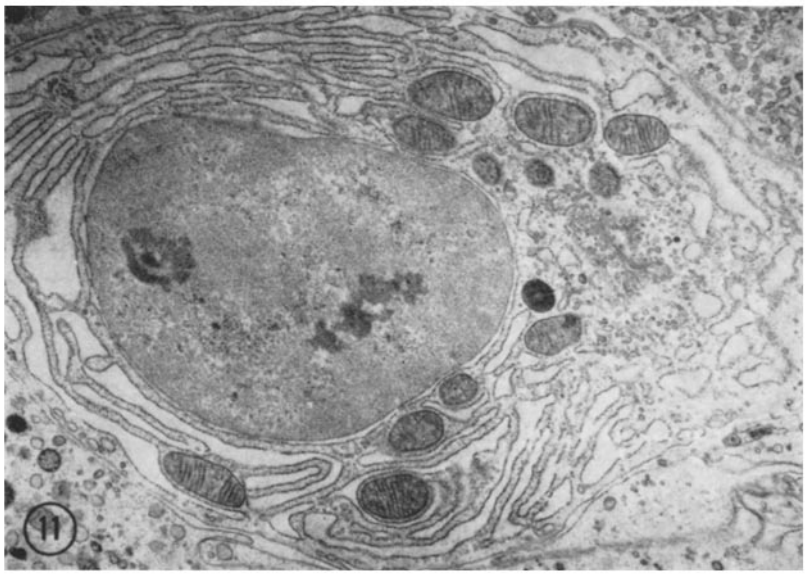

FIGURE 11 Electron micrograph illustrating the presence of a "typical" plasma cell, 17 days after immunization, in a chloramphenicol-treated animal. Note the elaborate Golgi apparatus and endoplasmic reticulum, the numerous RNP particles, and the presence of slightly electron-opaque amorphous material in the channels between the lamellae. Though well differentiated, only a few of these cells contained specific antibody and γ-globulin. Uranyl acetate and lead hydroxide. × 19,000.

duced the amount of protein synthesized. While protein synthesis was inhibited, RNA synthesis was unchanged. Withdrawal of CM resulted in a prompt restoration of protein synthesis, similar to the results of withdrawal of the antibiotic on antibody synthesis (5, 31).

Protein synthesis and, very likely, antibody synthesis in the spleen probably take place on polyribosomes. Essentially two classes of ribosomes can be demonstrated: those free in the cytoplasm and those bound to membranes of the endoplasmic reticulum. Talal and Exum (32) have shown a differential sensitivity of these two classes of ribosomes to CM. The membrane-bound ribosomes are inhibited more completely than free ribosomes, with respect to cell-free protein synthesis, and at lower concentrations. The synthesis of antibody protein is apparently associated with membrane-bound ribosomes (polyribosomes—plasma cell). A greater and more immediate effect would be anticipated on antibody synthesis by CM. In contrast, protein synthesis necessary for cellular replication and development occurs on ribosomes free in the cytoplasm and would not be expected to be as responsive to the drug.

Another consideration that may influence antibody synthesis is the delay in the formation of the endoplasmic reticulum and the attachment of ribosomes to the lamellae. Palade (33) and others (34) studied the relationship of the endoplasmic reticulum and the distribution of ribosomes with respect to protein synthesis in cells from a number of tissues. While ribosomes may occur free in the cytoplasm in rapidly dividing cells and in some "resting cells," they are usually attached to membranes in cells that synthesize "proteins for export." It has been shown that cells of the latter type treated to decrease or destroy endoplasmic reticulum have a lowered capacity for protein synthesis, even though there are a large number of ribosomal particles (34). The later development of endoplasmic reticulum in the potential antibody-producing cells observed in these experiments could result in a delay and decrease in antibody synthesis.

The critical time of administration of CM necessary to obtain inhibition of antibody synthesis suggests that the drug interferes with an important stage early. The administration of the drug must precede immunization or be given early in the inductive stage (3, 4), otherwise antibody synthesis is not markedly altered and the host is capable of

a nearly normal immune response with respect to circulating antibody. If sufficient synthesis of rRNA and mRNA occurs prior to giving CM, then the drug would have little effect. Presumably, sufficient assembly of functional synthetic units has taken place so that expression is seen. The prompt recovery of the immune system after the drug is withdrawn indicates that some of the essential machinery for antibody synthesis has been constructed in the presence of CM.

The authors are indebted to Mr. Caesar Moss and Mr. Virgil Mumaw for valuable technical assistance.

This work was supported by grants AM-07161 (to Doctors Schoenberg and Moore), H3952, and C4944 ASW from the United States Public Health Service. The work was performed during the tenure of a Research Career Development Award to Dr. Schoenberg from the United States Public Health Service.

Received for publication 29 July 1966.

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