

CORRELATION OF CELL DIVISION AND SPECIFIC PROTEIN PRODUCTION DURING THE COURSE OF LYMPHOID CELL DIFFERENTIATION

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SUMMARY

Mice were immunized with horseradish peroxidase and injected with [³H]thymidine. Popliteal lymph node cells were submitted to electron microscopic immunocytochemistry and high resolution autoadiography in order to correlate antibody production and ability to undergo cell division at various stages of lymphoid cell differentiation. Antibody synthesis started in blast cells and increased steadily until the mature plasma cell stage was reached. Thymidine incorporation was highest in blastoid cells and decreased continuously afterwards. Chromatin dispersion was found to be paralleled by thymidine incorporation. This observation and data of other authors seem to indicate that chromatin dispersion is a prerequisite for replication. Almost all mature plasma cells were devoid of thymidine incorporation. This confirms that they are end cells apparently unable to divide.

INTRODUCTION

Among cell differentiation systems, blast transformation of lymphoid cells and their maturation into plasma cells has been extensively studied. This cell system has the definite advantage that the specialized function of the involved cells, i.e. antibody synthesis and secretion, can be traced by means of various immunocytological techniques. The introduction of the immunoperoxidase method has permitted a thorough investigation of antibody production during the course of lymphoid cell differentiation at the ultrastructural level [10-12, 14].

On the other hand, isotopically labelled thymidine has been widely used to reveal DNA replication by autoradiographic methods and to follow the mitotic activity of cells at either the light microscopic or the electron microscopic level (see [4] for review).

Autoradiography and immunoperoxidase techniques can be combined as double tracer techniques at both the light microscopic [2] and the electron microscopic level. In the latter case, ultrastructural immunocytochemistry and high resolution autoradiography using tritiated amino acids proved to be a powerful tool to study synthesis, storage and secretion of specific antibodies in individual cells [2, 3].

In the present study, double tracer techniques combining immunocytology and autoradiography were used to reveal synthesis of specific antiperoxidase antibody and DNA replication in the course of lymphoid cell differentiation at the ultrastructural level. Thus, cell division and specific cell function, i.e. antibody synthesis, were followed and correlated at various stages of cell differentiation.

MATERIAL AND METHODS

Animals and immunization. C57BL mice were immunized with horseradish peroxidase (RZ3, Boehringer-Mannheim, BRD) in order to obtain a secondary immune response, according to a schedule described elsewhere [12].

Isotope injection. 48 h after the booster injection, the animals were injected with 1.6 mCi of [6-³H]thymidine, spec. act. 15 Ci/mmol (CEA, Saclay, France) and sacrificed 2 h later.

Tissue preparation. Popliteal lymph nodes were dissected out of animals and fixed for 24 h in 4 % formaldehyde, freshly prepared from paraformaldehyde (Merck, BRD) in 0.2 M cacodylate buffer, pH 7.2, then washed with several changes of the buffer solution for at least 24 h. Small tissue fragments and also 40 μm thick frozen sections, prepared in a Dittes-Duspiva cryostat, were used for immunocytochemical incubation as described elsewhere [10, 12].

Immunocytochemistry. Antibody-containing sites in cells were revealed as previously described [14]. Briefly, cells were incubated in peroxidase, 1 mg/ml in cacodylate buffer for 24 h at room temperature, washed three times for 10 min, incubated in 3,3'-diaminobenzidine (Merck), 0.5 mg/ml in 0.2 M Tris-HCl buffer, pH 7.2, containing 0.01% hydrogen peroxide [7], washed in cacodylate buffer, postfixed in 2% osmium tetroxide in cacodylate buffer for 1 h, dehydrated in ethanol and embedded in Epon. Controls for the specificity of the immunocytochemical reactions were obtained as usual [10, 14].

High resolution autoradiography. The method employed in the present paper is the same as in previous papers [2] and has been extensively described elsewhere [5]. In brief, Ilford L4 emulsion was applied on single grids using the gold interference coloured zone of the emulsion film in a platinum loop. After 3 months of exposure, the autoradiographs were developed by the gold latensification method followed by a phenidon containing developer.

The grids were examined in a Siemens Elmiskop IA, operating at 80 kV with 50 μm objective apertures.

Quantitation. A total of 1477 lymphoid cells were randomly counted and computed according to the presence of radioactivity in their nuclei and localization of specific antiperoxidase antibody in cytoplasmic compartments. Lymphoid cells and their differentiation products were classified following previously published criteria [10, 11].

Cells were regarded as radioactive when nuclei were covered by a minimum number of 10 developed silver grains (fig. 2). This criterion was particularly useful in the present study because specifically labelled cells displayed usually a high number of grains (figs 3-5), whereas unlabelled cells such as typical small lymphocytes showed only background labelling of <3 grains/100 μm^2 .

When cells had cytoplasmic organelles (perinuclear space, rough surfaced endoplasmic reticulum, Golgi apparatus) which contained specific antiperoxidase antibody, these cells were considered as antibody-producing cells (figs 4, 6, 7).

RESULTS

Results are summarized in table 1 where total cell counts are tabulated with respect to specific antibody staining, radioactivity and degree of cellular differentiation. Respective percent ratios are shown in tables 2, 3 and fig. 1.

Table 1. *Total cell counts*

| | Lymphocytes | Blastoid | Blast | Immature plasma C | Mature plasma C | Total |
|--------------------------------|-------------|----------|-------|-------------------|-----------------|-------|
| <i>Antibody-positive cells</i> | | | | | | |
| Radioactive | 0 | 0 | 6 | 28 | 3 | 37 |
| Not radioactive | 0 | 0 | 0 | 24 | 163 | 187 |
| <i>Antibody-negative cells</i> | | | | | | |
| Radioactive | 7 | 86 | 145 | 60 | 6 | 304 |
| Not radioactive | 721 | | 66 | | 162 | 949 |
| Total | | | | | | 1 479 |

Cell counts were computed according to the presence of radioactivity and antiperoxidase antibody at each stage of lymphoid cell differentiation as judged from ultrastructural criteria.

Table 2. *Percent ratios of antibody staining cells which incorporate [³H]thymidine*

| | Lymphocytes | Blastoid | Blast | Immature plasma C | Mature plasma C |
|-----------------|-------------|----------|-------|-------------------|-----------------|
| Radioactive | — | — | 100 | 53.8 | 1.8 |
| Not radioactive | — | — | 0 | 46.2 | 98.2 |
| Total | — | — | 100% | 100% | 100% |

Table 3. *Percent ratios of non antibody staining cells which incorporate [³H]thymidine*

| | Lymphocytes | Intermediate stages | Mature plasma cells |
|-----------------|-------------|---------------------|---------------------|
| Radioactive | 1 | 75 | 3.5 |
| Not radioactive | 99 | 25 | 96.5 |
| Total | 100 | 100 | 100 |

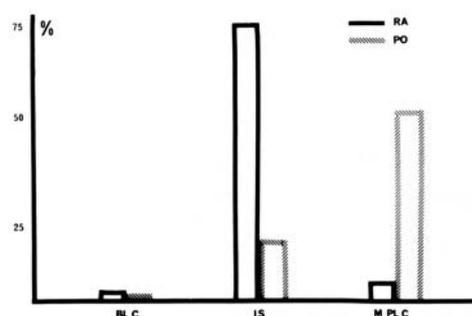


Fig. 1. Diagram illustrating the respective variations of [³H]thymidine incorporation and specific antibody production at various stages of lymphoid cell differentiation. Black column, % radioactive cells; shaded column, % antibody-forming cells. *BLC*, blast cells; *IS*, intermediate stages; *MPLC*, mature plasma cells.

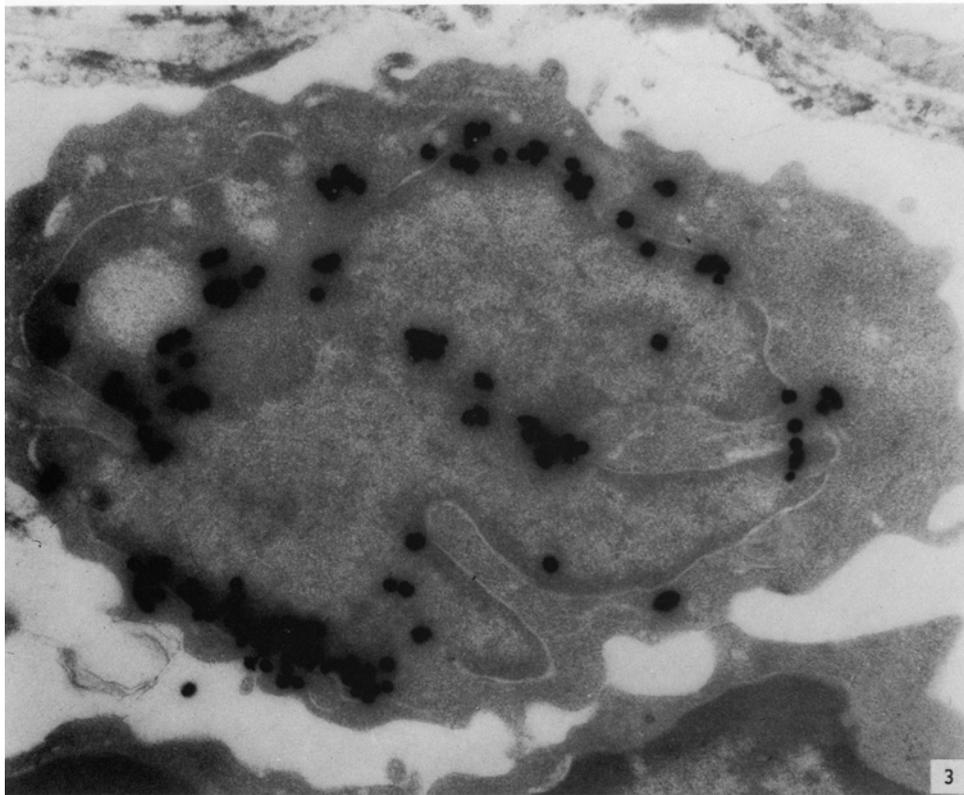
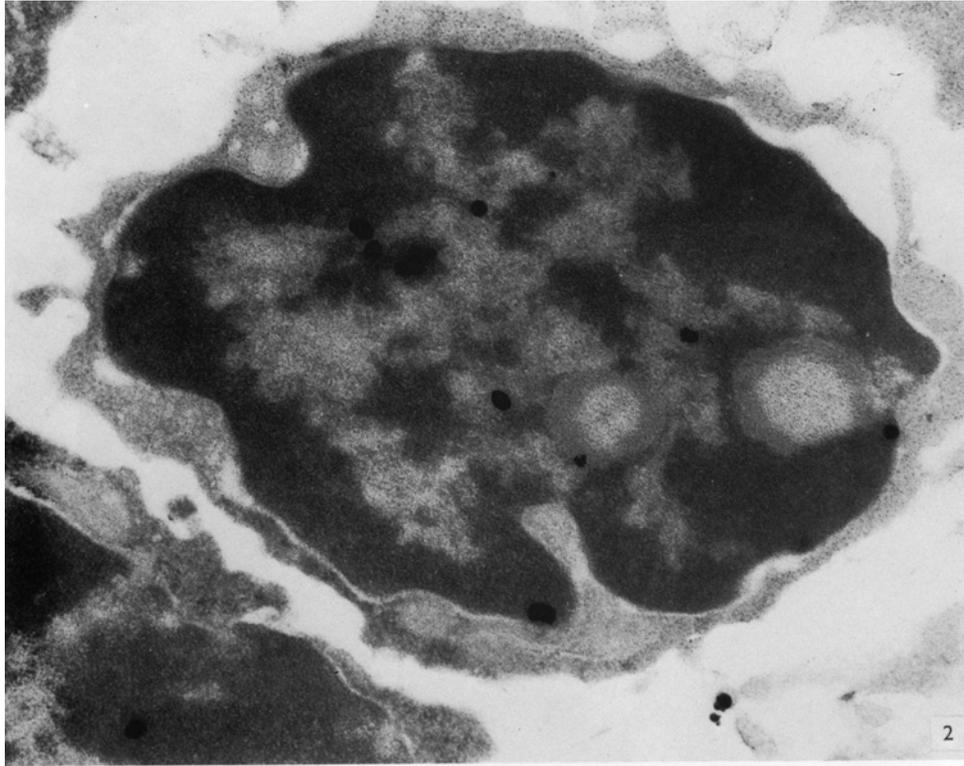


Fig. 2. Small lymphocyte as defined by high nuclear/cytoplasmic ratio. Absence of ergastoplasmic cisternae; note highly condensed chromatin. The lymphocyte illustrated was one of the few with more than 10 grains, and therefore counted as radioactive. x 24 500.

Fig. 3. Blastoid cell with still poorly developed cytoplasmic components. Large nucleus showing several invaginations. Chromatin begins to disperse. None of these cells were found to produce specific antibody, while most of them incorporated thymidine. x 17 500.

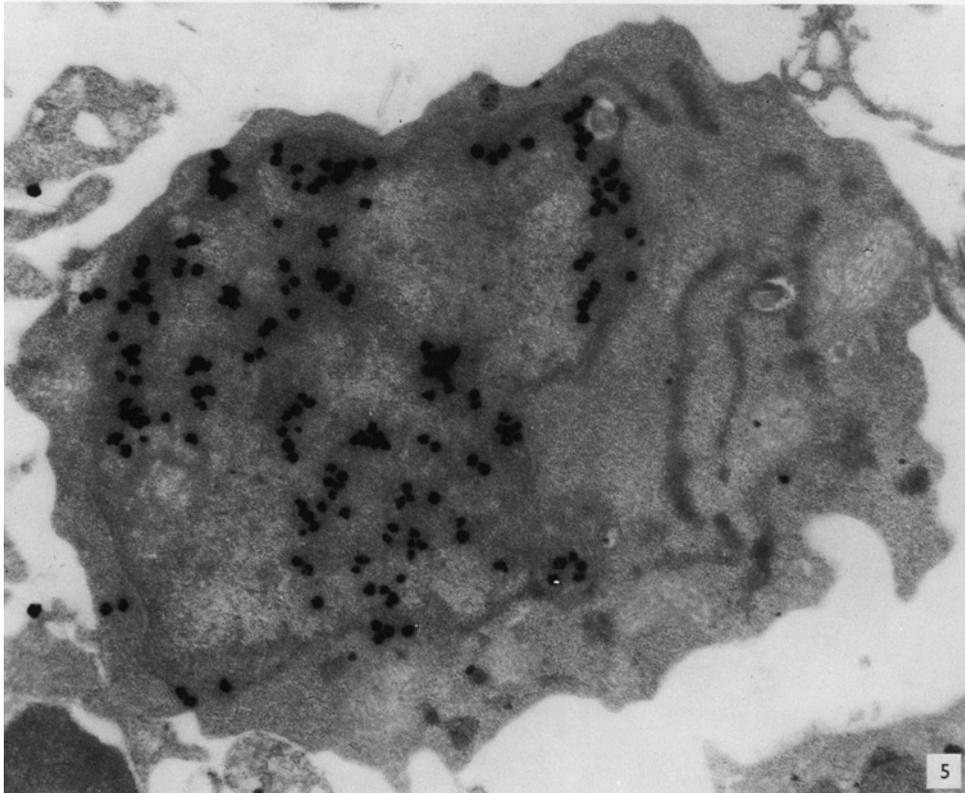
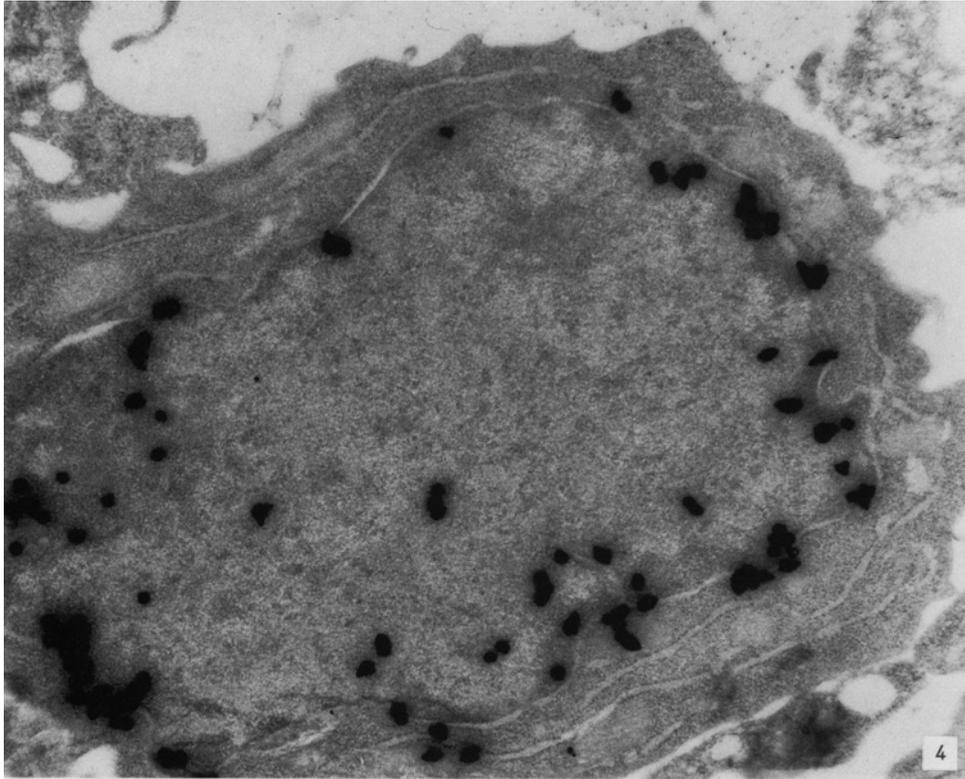


Fig. 4. Blast cell producing antiperoxidase antibody in few ergastoplasmic lamellae. Chromatin is fully dispersed and heavily labelled with [^3H]thymidine. x 17 500.

Fig. 5. Blast cell with same morphological features and autoradiographic labelling as in fig. 3. No antibody staining. x 17 500.

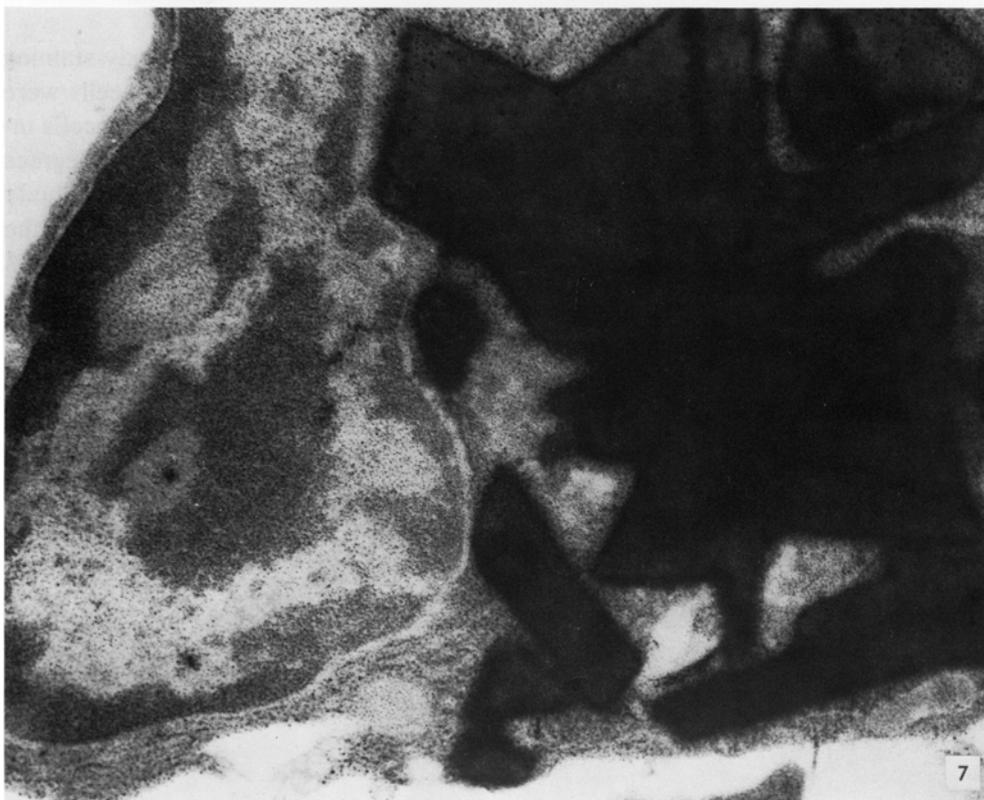
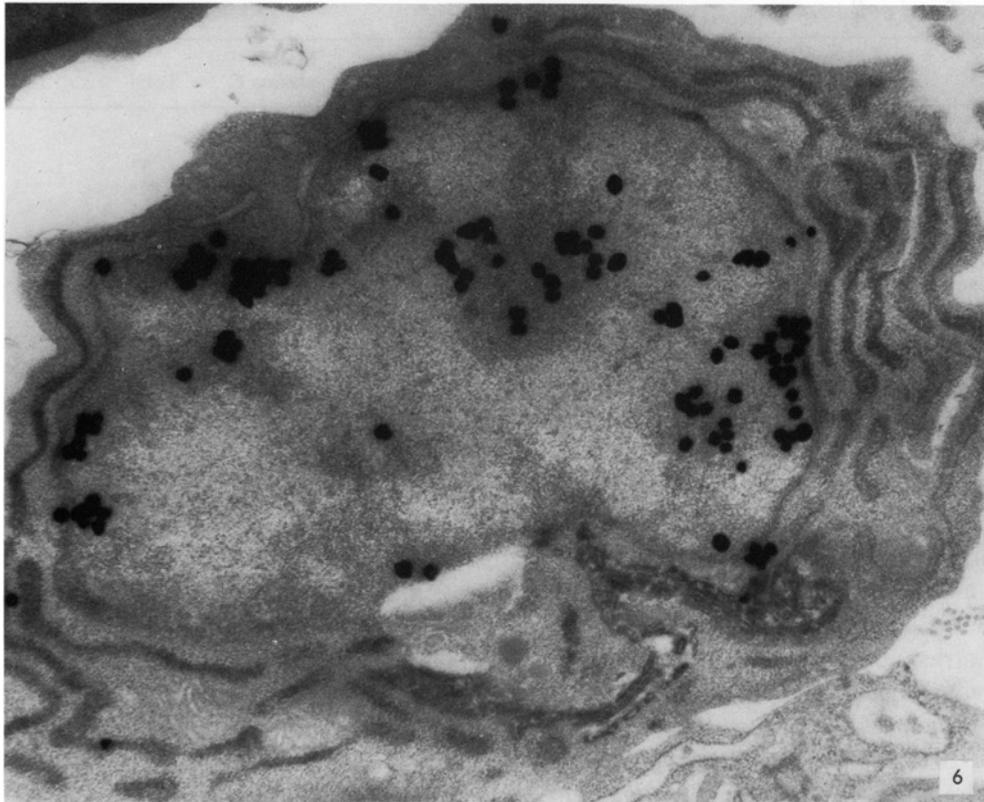


Fig. 6. Immature plasma cell exhibiting specific antibody in rough ER and Golgi apparatus. Note heavy labelling of the nucleus. x 17 500.

Fig. 7. Mature plasma cell with large antibody containing Russell's bodies. The majority of mature plasma cells, with or without Russell's bodies were devoid of thymidine uptake. x 23 000.

Small lymphocytes were usually unlabelled, and less than 1 % were specifically labelled (fig. 2).

In contrast, blastoid cells were found to be heavily labelled by [³H]thymidine, but none of them were stained for antibody (fig. 3). Blast cells were observed to be largely involved in DNA replication (figs 4-5) and some of them (fig. 5) already synthesized antibody. Specific antibody-producing blast cells were always labelled by [³H]thymidine. When blast cells were compared with morphologically more differentiated cells (fig. 6), antibody synthesis increased markedly, while incorporation of thymidine decreased (table 1).

The majority of mature plasma cells failed to incorporate labelled thymidine, whether involved in specific antibody synthesis or not (fig. 7). However, 1.8% of antiperoxidase antibody positive plasma cells were actually incorporating thymidine, which is a small but significant number (table 2).

Percentage ratios of antibody-staining and [³H]thymidine-incorporating cells were plotted for the whole population of cells investigated according to three main degrees of differentiation (fig. 1). Thus, antibody formation increased continuously with the degree of differentiation, while the DNA replicating process was restricted to intermediate stages, excluding both small lymphocytes and mature plasma cells.

DISCUSSION

Attempts have been made recently to compare lymphoid cell differentiation with thymidine incorporation by means of electron microscopic autoradiography in order to investigate the ability for cell division at various stages of differentiation and also the localization of the replication sites. Lymphocyte stimulation by phytohemagglutinins is one of the most interesting models in which initiation of replication has been thoroughly investigated [15, 16, 21]. Unfortunately, no correlation of those findings with specific protein synthesis is possible in such experiments. Rosette-forming cells can be labelled with thymidine [8], but this immunological technique most likely reveals not only actively engaged antibody-producing cells, but also preformed surface receptors on immunologically competent cells. Finally, thymidine incorporation in *Salmonella* stimulated cells was recently compared with antiperoxidase activity [9] but visualization of antibody formation in the very cells that incorporated thymidine could not be obtained by this method. The present study adds new information to the data brought by these three studies inasmuch as comparison of thymidine incorporation and antibody synthesis was carried out in individual cells by means of simultaneous immunocytochemical and ultrastructural autoradiographic labelling.

Thus, the main goal of this study was to correlate the ability of cells to undergo cell division and the capacity to produce specific antibody at each stage of differentiation during the immune response as defined by ultrastructural criteria. Functional classification of differentiating lymphoid cells has been proposed for the immunoperoxidase system and compared with other experimental conditions [10-12]. According to this classification, small lymphocytes are precursors of a line of differentiating cells which become involved in antibody production: blastoid cells, blast cells, immature and mature plasma cells. One of the most striking changes in ultrastructure of cells during the course of differentiation is the state of condensation of the chromatin. These changes are apparently related to the degree of chromatin activation in terms of replication and transcription (see [4] for review) as shown in

experimental situations [6, 13]. The present study allowed the correlation of chromatin changes with cell division and antibody production, thus, the course of lymphoid cell differentiation can be described as follows.

Small lymphocytes are resting G₀ cells, not actively engaged in specific antibody synthesis nor in DNA replication, their chromatin displaying a highly condensed appearance. Then, blastoid cells become strongly active in premitotic DNA replication, their chromatin undergoes marked dispersion, however, they are still inactive in terms of specific protein synthesis. Antibody formation starts with the appearance of typical blast cells where the chromatin is almost entirely dispersed and the blast cells incorporate [³H]thymidine in large amounts. The following stage is the immature plasma cell where antibody production becomes prominent while DNA synthesis decreases markedly. The final stage is the mature plasma cell whose main activity is devoted to specific antibody synthesis, whereas DNA replication comes to a standstill which is in agreement with recondensation of chromatin.

The occurrence of blast cells, immature plasma cells and mature plasma cells without staining for antiperoxidase antibody, although displaying the same amount of radioactivity as antiperoxidase antibody positive cells is not surprising, since it is known that lymphoid tissues contain a variety of immunocompetent cells sensitive to other antigens than those used in the present study.

From our results it can be concluded that the great majority (though not all) mature plasma cells are unable to incorporate thymidine, thus confirming that most mature plasma cells are so-called end cells devoid of mitotic activity. Because the life span of those cells seems to last some time [10, 11], parts of the synthesized antibody in the cytoplasm may result from storage. However, mature plasma cells are also the site of further antibody synthesis since ergastoplasmic reticulum filled with antibody could be shown to incorporate tritiated amino acids [22] as well as carbohydrates [3]. These observations indicate a clearcut difference between mature and immature plasma cells, the latter still able to undergo cell division.

The importance of the small percentage of mature plasma cells which incorporate [³H]thymidine is difficult to assess. Labelling could be due to DNA synthesis unrelated to cell division [17, 18, 20], and possibly reflecting DNA repair [19]. This is supported by the observed labelling of 1 % of the small lymphocytes in our experiments. If the autoradiographic labelling was not related to DNA repair but to premitotic DNA synthesis, this suggests that part of the immune response can be carried out through mature plasma cell division without requiring full-line cell differentiation. However, as long as no evidence is provided to support this hypothesis, the present data strongly suggest that the term 'mature' plasma cell should be restricted to differentiated plasma cells that do not incorporate thymidine.

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