

## THE LIFE SPAN OF IgA PLASMA CELLS FROM THE MOUSE INTESTINE\*

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In conventionally reared Swiss mice, the first plasma cells appear after the second week of life, increase rapidly during the third week, and by 1 mo the number of intestinal plasma cells approach those found in adult mice (1). The plasma cell population of the lamina propria of the mouse intestine belongs almost exclusively to the IgA class (2). The development of this cell population depends on environmental antigens which are ingested and reach the gastrointestinal mucosa with the diet. Thus, germfree mice have a poorly developed intestinal plasma cell system which can be stimulated by placing these mice in a conventional habitat (2).

In the present paper, we report studies bearing on the life span of intestinal IgA cells under physiological conditions of stimulation. By treating mice with tritiated thymidine (TTH)<sup>1</sup> during the critical period after birth when the plasma cells are rapidly appearing, essentially 100% of intestinal plasma cells were labeled. A technique combining immunofluorescence with autoradiography allowed us to scan large numbers of IgA cells for nuclear labeling with tritium. The half-life of IgA cells was estimated from the rate of disappearance of tritiated IgA cells.

### *Materials and Methods*

*Mice.*—CBA mice originally obtained from Jackson Laboratories (Bar Harbor, Me.) were bred in our animal facilities. The mice were kept free from ecto- and endoparasites. Pregnant mice were individually caged and fed with a standard laboratory mouse diet and water ad libitum. Litters were weaned when 22-days old.

*Antisera.*—The preparation and specificity control of the fluorescein-labeled goat antirabbit IgG and rabbit antimouse immunoglobulins will be described in detail in a subsequent publication.<sup>2</sup> Briefly, antisera to mouse immunoglobulins were made in rabbits with immunopre-

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<sup>1</sup> Abbreviations used in this paper: TTH, tritiated thymidine.

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precipitated antigen. Precipitin bands were obtained by immunoelectrophoresis, cut out, and injected with complete Freund's adjuvant. Antigenic mixtures used in the immunoelectrophoresis step were whole mouse colostrum for the preparation of antimouse IgA antisera; IgM-rich fraction (from pooled normal mouse serum obtained by gel chromatography) for the anti-IgM antisera; and papain-digested IgG (obtained by starch block electrophoresis of pooled normal mouse serum) for the anti-IgG antisera. Antisera to IgA and to IgM were absorbed with pooled newborn serum to render them monospecific.

*TTH*.—Sterile, nonpyrogenic aqueous solution of TTH with a specific activity of 26.6 Ci/mM (ICN Corp., Chemical & Radioisotopes Div., Irvine, Calif.) was diluted with sterile saline and injected intraperitoneally with a 26 gauge needle. Immediately after sacrificing the mice, the proximal centimeter of the small bowel was removed, fixed in 10% buffered formaldehyde solution for 2 h at 4°C, and then left in 30% sucrose overnight. Cryostat sections, 4  $\mu$ m thick, were prepared on gelatin-coated slides. Indirect fluorescent staining (3) was carried out by incubation with unlabeled rabbit antimouse IgA antiserum, washing, incubation with fluorescein-labeled goat antirabbit antiserum, and washing in 95% ethyl alcohol. Autoradiography (4) was performed by dipping the slides in Kodak NTB<sub>2</sub> emulsion diluted 1:1 with distilled water and exposing in light-proof boxes at 4°C for 1–4 wk. Development was carried out with Kodak D-19 for 2 min at 25°C and fixation with Kodak acid fixer for 3 min. Slides were mounted with buffered glycerin and sealed with clear nail polish. They could be stored for months at 4°C without appreciable loss of specific fluorescence. A similar procedure was used to process spleen sections for IgA, IgG, and IgM cells.

Slides were examined under dark field with a Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with both ultraviolet and standard illumination. Pictures were taken with an Orthomat camera. For control purposes, standard autoradiography was done on sections stained with hematoxylin-eosin, methyl green-pyronine, and May-Grünwald-Giemsa.

## RESULTS

Preliminary experiments were performed to test the feasibility of the immunofluorescence-autoradiography technique and to adjust methodological details such as the best exposure time for the autoradiographic slides. To assess reliability and to recognize possible artifacts, the combined technique was compared with the standard autoradiographic method. Although a good general correlation was found, it was easier and more accurate to identify both the labeled and unlabeled plasma cells by the combined technique.

In order to label the maximum number of plasma cells, variables studied in pilot experiments were: doses of TTH, frequency of the injection, starting age, and total number of days to be covered by the treatment. Data selected from these experiments are shown in Table I.

In conventional Swiss mice, the first intestinal IgA cells appear after the second week of age and then rapidly increase in number until day 35 when they become a major cellular component of the lamina propria (Figs. 1 *a* and *b*). Based on these data 15  $\mu$ Ci of TTH was injected every 6 h from day 15 to day 35 of age. 1 day after the last injection, the first group of three mice were killed by cervical dislocation. Similar groups of three were sacrificed at 2, 4, 5, 10, 15, 20, 35, 45, 60, and 90 days. In addition to the use of the CBA inbred strain of mice, litters of uniform size (six to eight animals) were chosen and the three

TABLE I  
*Number of IgA Cells Labeled with Tritium after Different Schedules of TTH Administration*

Group no.	TTH daily dose $\mu\text{Ci}$	Period of TTH injections (days of age)	Day of sacrifice	Tritium-labeled plasma cells (mean $\pm$ SE) %
1	10	15-25	26	23.7 $\pm$ 2.8
2	60	20-25	28	20.7 $\pm$ 1.9
3	60	20-30	33	72.0 $\pm$ 2.7
4	60	15-35	36	93.3 $\pm$ 0.4
5	60	15-35	36	98.7 $\pm$ 0.2

Combined immunofluorescent-autoradiography technique. Autoradiograph exposed for 7 days (groups 1-4) or 21 days (group 5). Each test group includes three mice. In groups 2-5 the 60  $\mu\text{Ci}$  total daily dose was divided into four equal doses given every 6 h.

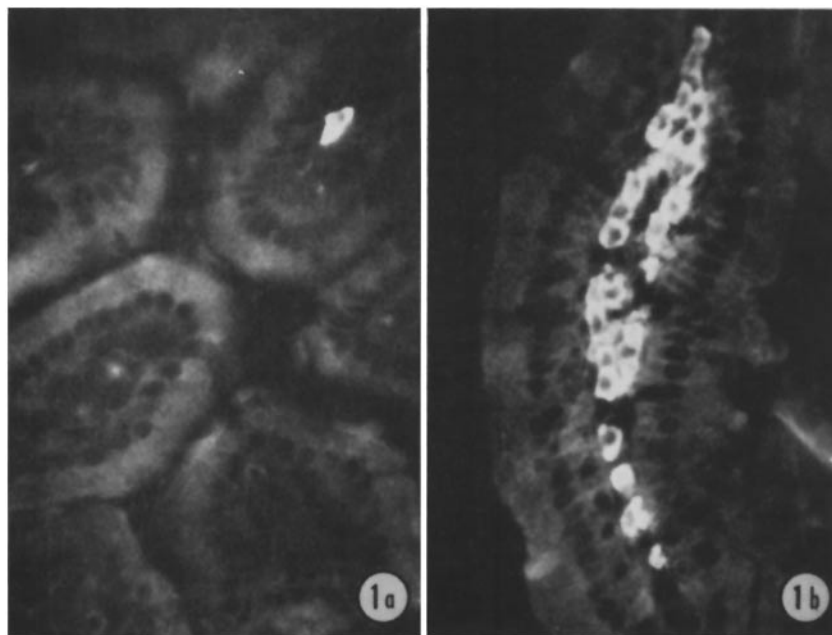


FIG. 1. (a) Transverse section of several intestinal villi from a 15-day old mouse. Many villi were scanned and only one IgA plasma cell was found. (b) Longitudinal section of a villus from an adult mouse illustrating the large number of IgA plasma cells present in the intestinal mucosa of conventional animals. Fluorescent photomicrographs.  $\times$  250.

animals in each test group were taken at random from different litters to compensate for interlitter variability. There was no selection according to sex. Variations inherent to the autoradiographic procedures were diminished by processing all slides simultaneously. To detect the IgA cells, duodenal sections

were stained with fluorescein-labeled antiserum as described in Materials and Methods. The slides were observed first with ultraviolet light and IgA-fluorescent cells identified. Then, visible light was added and silver grains, indicative of tritium incorporation into the cell, were seen as bright dots. IgA cells with five or more silver grains over their nucleus were considered to be positively labeled (see Fig. 2). Background counts were less than one grain in an area equivalent to one plasma cell. For each mouse studied, three different



FIG. 2. High magnification view of two IgA plasma cells in the intestinal lamina propria. The tritium-labeled plasma cell on the top shows many silver grains over the nucleus. The other plasma cell is unlabeled. Mouse killed 10 days after the last TTH injection. Combined immunofluorescent-autoradiographic technique.

sections were made and a minimum of 100 IgA cells were counted, single blind, in each section.

The results are summarized in Fig. 3, which shows the disappearance rate of tritium-labeled IgA-containing cells. Each point on this graph represents at least 900 IgA cells on which the percent of tritium-labeled cells were computed.

Analysis of the curve reveals a lag period (first two points) before tritium-labeled IgA plasma cells begin to disappear. The lag period could be explained by the initial replacement of the older plasma cells by young plasma cells originating from heavily labeled precursors. After the lag period, the next five points follow a decreasing straight line, indicating that during this time period the percent of tritium-labeled IgA plasma cells is decreasing approximately as

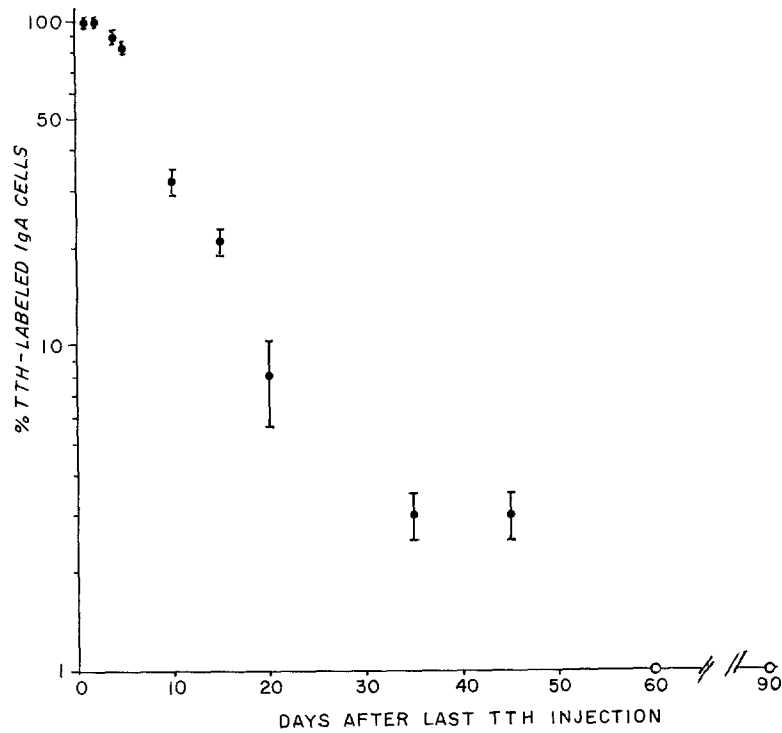


FIG. 3. Semilogarithmic plot of the disappearance rate of tritium-labeled IgA-containing cells. Each dot represents the mean value and the vertical bar the corresponding standard error obtained for a group of three mice. The open circles (day 60 and 90) indicate zero tritium-labeled IgA cells.

an exponential function of time. After these points, the curve appears to bend to the right. A curve of this shape would be consistent with the hypothesis that there are at least two types of labeled cells which differ in their half-life. If this is indeed the case then the predominant and shortest-lived type of cell can be approximated from the five points between day 4 and day 20 to have a half-life of 4.7 days. There are not enough points beyond day 20 to calculate the half-life of the second type of cell which would represent a minor population of longer lived cells. However, since there were no detectable tritium-labeled IgA plasma cells remaining at day 60, if there is a second type of cell, its half-life would be significantly less than 60 days.

The small number of plasma cells normally present in unstimulated mice spleens of young age prevented us from doing a parallel study on spleen plasma cells similar to the one carried out with gut IgA plasma cells. However, it was possible to investigate the maximum survival time of IgM, IgG, and IgA plasma cells. The last labeled plasma cells were seen in the spleen on day 45 after the last injection of TTH.

Mice killed at 24 h showed all the nuclei of the intestinal epithelial cells heavily labeled with tritium. 10 days after TTH administration labeled epithelial cells (with five or more grains over the nucleus) were no longer detected, but 32% of the IgA plasma cells were still labeled (see Fig. 3). By day 60, tritium-labeled plasma cells were no longer seen, while many nonplasma cells were still clearly labeled, about one-third of them heavily, i.e., with more than 10 silver grains. By conventional autoradiographic procedures and stainings, most of the nonplasma cells showed the morphology of histiocytes, reticular cells, and lymphocytes. These observations on the labeling behavior of the epithelial cells and of the nonplasma cells intrinsic to the intestinal lamina propria offer internal controls pointing to the validity of our observations on the renewal rate of the plasma cell population.

Two males and three females from the main experiment were not killed and at 10 mo of age they appeared healthy. They were able to mate and capable of rearing normal offspring, indicating that at least in this regard there was no detectable biological damage by the TTH dose employed.

#### DISCUSSION

In order to label essentially 100% of the plasma cell population of the intestine it was necessary to give a relatively high dose of TTH at short intervals, e.g. 15  $\mu$ Ci every 6 h from day 15 to day 35, and to expose the autoradiographic slides for at least 21 days. These requirements can be understood if it is considered that the continuous and varied stimulation produced by the environmental antigens results in highly asynchronous waves of mature plasma cells and that all dividing cells will be labeled only if the interval between injections is less than the S period of the cell cycle.

Our results indicate that the half-time disappearance rate of the majority of IgA cells from the mouse intestinal mucosa is 4.7 days. It should be stressed that this is an estimate of the renewal rate within the intestinal compartment rather than the true life span of the IgA cells.

It is pertinent to mention some factors which could affect the figure of 4.7 days as being representative of the actual half-life of the IgA plasma cells. The results of the labeling pattern of the intestinal epithelial cells suggests that there is no significant reutilization of the radioactive marker. Other factors relating to the kinetics of the IgA plasma cells as a proliferating open cell compartment are as follows.

(a) With our radiolabeling approach, the apparent half-life of the IgA plasma cells will be increased by the arrival of newly secreting IgA cells, labeled with TTH earlier at a precursor stage. This effect would be expected to be more noticeable in mice sacrificed at early days and is probably responsible for the initially slow decay in percentage of the TTH-labeled IgA cells (lag period) shown in Fig. 3. For this reason the first two points of the curve were not included in the computation of the half-life. This may also be one of the reasons for the gradual decline in the degree of nuclear labeling seen with time although

the most likely explanation is the more rapid rate of disappearance of the most heavily labeled older cells.

(b) Prolongation of the apparent half-life will be occasioned by division of TTH-marked young plasma cells. Some cells start synthesis and release antibody while still capable of division (5). However, the vast majority of the cells with the morphology of mature plasma cells do not divide, most probably being the highly specialized end stage of a particular lymphoid cell lineage (6). It should be pointed out that the definition of a plasma cell used in this study is a functional one. We considered plasma cells as those cells with a cytoplasm clearly stained by the immunofluorescent technique. Using this criterion, most of the cells studied were mature plasma cells but a small number of young plasma cells and plasmablasts were also included.

(c) Migration of IgA cells to other tissues as suggested by some experiments (7, 8) or metamorphosis to other lymphoid cells (9) will give a spurious shortening of the calculated half-life, although these are unlikely possibilities in our opinion.

(d) The intestinal plasma cell population is still being expanded at 35 days of age, since the number of plasma cells per villi increases for several weeks more before reaching a steady state. In this situation, the calculated half-life will also be shortened if unlabeled precursors differentiate to plasma cells. However, the overall linearity of the curve suggests that this effect is probably not of great importance.

Most previous studies on the life span of plasma cells have been carried out on lymph nodes of mice or rats after antigenic stimulation. These studies suggest that plasma cells producing specific antibodies to the injected antigen persist in the lymph node with a half-life of 8–12 h (10) to 2 days (11) compared with 4.7 days in our studies. There are certain differences between these studies and ours. Because of the abundance of IgA cells in the normal intestinal lamina propria there was no need for additional antigenic stimulation and, therefore, the population of IgA cells in our study represents a physiological response to the multiple and protracted stimulation produced by the normal antigenic load. It could be speculated that quantitative and/or qualitative antigenic variations may result in plasma cell populations which differ in their half-life. In support of this concept is a report that plasma cells from rat lymph nodes have a half-life of 4 days in unstimulated animals but only of 2 days in the antigenically stimulated rats (6). An analogy can be drawn with the median life of the intestinal epithelial cells, which is prolonged in germfree mice compared with conventional mice (12). In the previous studies on lymph node plasma cells the immunoglobulin class of the cells were not determined and it may be that IgA cells have a different half-life than cells producing other classes of immunoglobulin. Alternatively the intestinal site could be associated with cells having a longer average life span than cells located in peripheral sites, such as spleen and lymph nodes. Finally, we studied the plasma cell population during the

neonatal period, which may differ from the population of adult mice in its renewal rate.

One additional reason for choosing the neonatal period was the speculation that this could be a propitious time during development to build up a subpopulation of long-lived plasma cells, perhaps as a mechanism of immunological memory. The type of memory we are referring to here would be mediated by the end product, i.e. antibody which could possibly mediate memory by forming specific complexes which would focus and preserve the antigen at relevant sites, such as the dendritic macrophages of the peripheral lymphoid organs. In fact, some studies have described plasma cells capable of living for periods of 6 mo or longer (13-15). In one study, 6 mo after TTH injection 8% of all plasma cells were reported as still being labeled (13). However, we found only 3% tritium-labeled IgA plasma cells 1 mo after isotopic labeling and none after 2 mo. Confidence in our conclusions is given by the greater specificity in the identification of plasma cells furnished by the immunofluorescence technique and the substantially larger sample size investigated. Our observations indicated that plasma cells do not play a significant role in long-term memory and suggest that the persistence of antibody production after antigenic stimulations depends on the recruitment of new immunocompetent cells.

The curve depicted in Fig. 3 suggests the possibility of a minor population of cells having a longer half-life. Since no labeled plasma cells are detectable after 2 mo, the half-life of this presumed "long-lived" population would be considerably less than 60 days and could therefore not be of sufficient duration to explain long-term memory. Scanning of spleen slides for IgM and IgG plasma cells with the same technique used for IgA cells showed that the last labeled cells were seen on day 45 and suggests that similar conclusions can also be applied to the other main immunoglobulin classes of plasma cells in peripheral lymphoid tissues.

#### SUMMARY

Tritiated thymidine was injected during the neonatal period into conventional CBA mice. The disappearance rate of tritium-labeled IgA plasma cells was followed by a technique combining immunofluorescence with autoradiography which permitted the identification of the immunoglobulin class of plasma cells with labeled nuclei. In this way, it was possible to calculate that the major population of intestinal IgA plasma cells have a half-life of 4.7 days under physiological conditions of stimulation. It was also found that the maximum life span of both intestinal IgA plasma cells and the IgA, IgG, and IgM plasma cells from the spleen is of the order of 7-8 wk.

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