A CYCLICAL APPEARANCE OF ANTIBODY-PRODUCING CELLS AFTER A SINGLE INJECTION OF SERUM PROTEIN ANTIGEN

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The role of antibody in the regulation of the immune response has become a major issue only in the past few years, even though it has been known for over half a century that mixing antigen with excess antibody can suppress the antibody response (1). A regulatory role of antibody in the immune response was first suggested by Uhr and Baumann (2) in 1961, based on experiments showing that passive antibody inhibits the immune response. Although it has clearly been demonstrated by numerous workers (cited in reference 1) that passive antibody can interfere with the expression of an immune response, little information has been obtained which demonstrates the role of antibody produced during an antibody response on the control of that same response. The best evidence supporting the latter point is the enhancement of the immune response after removal of circulating antibody (3, 4). Such information strongly suggests that circulating antibody plays an important role in regulation of the immune response once it has been initiated. In the case of antigens which persist in the body tissues, antibody may also regulate the immune response over longer periods of time. In fact, it has been demonstrated that the immune response to lipopolysaccharide from Escherichia coli shows a cycling phenomenon which appears to be controlled by the level of circulating antibody (5). Thus, while both normal catabolism of antigen and inhibition by antibody may terminate the immune response to antigens that are readily catabolized, it appears that antibody alone is probably responsible for the regulation of the immune response to antigens such as lipopolysaccharides which are poorly catabolized and persist in tissues for long periods of time.

The present paper shows that injection of the aggregated form of serum protein antigens in rabbits results in a cyclic appearance of antibody-producing cells which appears to be synchronized. Experiments will be presented which both implicate locally produced antibody in regulation and delineate some of the possible cellular events that may be involved.

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Materials and Methods

Animals.—New Zealand White rabbits weighing 2.5-3.0 kg were used in all experiments.

Antigens.—Cohn fraction II from human sera (HGG) was obtained through the courtesy of the American Red Cross National Fractionation Center. The IgG fraction (HuIgG) was isolated by diethylaminoethyl (DEAE)-cellulose chromatography on columns equilibrated with 0.01 M phosphate buffer, pH 8.0. Pooled turkey sera was precipitated (twice) with ammonium sulfate \((\text{NH}_4)_2\text{SO}_4\) at a final concentration of 35% and the IgG fraction (TuIgG) was isolated by filtration on G-200 Sephadex essentially according to the method described by Benedict (6). Both immunoglobulins were heat aggregated at 63°C for 25 min (7).

Radioisotope Studies.—Proteins were trace labeled by the chloramine-T method as modified by McConahey and Dixon (8). Radioactivity was measured in a gamma scintillation counter with a sodium iodide crystal.

X Radiation.—Two opposing 220 kV Picker machines, 160 cm apart, were used to deliver 500 R whole body irradiation (50-55 R/min) to rabbits placed in a leucite container midway between the sources. The machines were operated at 210 kV, 15 mA, with an inherent filter of 0.25 mm Cu and 1 mm Al.

Hemolytic Plaque Assay.—Spleens and mesenteric lymph nodes were removed from rabbits immediately after exsanguination, trimmed of fat, and teased apart in balanced salt solution (BSS) buffered to pH 7.0 with ethylenediaminetetraacetic acid (EDTA) at a final concentration of 0.02 M. Spleen fragments were teased through 90-mesh wire screens (Newark Wire Cloth Co., Newark, N. J.) and filtered through nylon monofilament cloth (Tobler, Ernst, Traber, Inc., New York). Cells were washed one time in EDTA-BSS and three times in BSS (all centrifugations at 250 g for 10 min at 4°C) before suspending to a concentration of 2-4 \(\times\) 10^7 cells/ml.

Blood was collected steriley by cardiac puncture into tubes containing EDTA, penicillin, and streptomycin at final concentrations of 0.02 M, 100 U/ml and 100 \(\mu\)g/ml, respectively. The blood was mixed in a graduated cylinder with an equal volume of 3% gelatin (Matheson, Coleman and Bell, Los Angeles, Calif.) dissolved in EDTA-BSS. The cells were allowed to settle 30 min at 37°C, and the supernate was aspirated as closely as possible to the red blood cell interface. The supernate was centrifuged at 250 g for 10 min at 37°C, washed one time in EDTA-BSS also at 37°C, and treated with 10 ml 0.83% ammonium chloride for 5 min at room temperature (to lyse contaminating red blood cells) before centrifugation at 4°C. Cells were washed three times in BSS and suspended to a concentration of 2-4 \(\times\) 10^7 cells/ml. All procedures for isolating cells from blood were carried out with siliconized glassware.

Antibody-forming cells were measured by a modification (9) of the Jerne plaque assay (10). Goat red blood cells (GRBC) (Colorado Serum Co., Denver, Colo.) were used as indicator cells. GRBC-absorbed HGG or GRBC-absorbed turkey gamma globulin, prepared by 2X precipitation of turkey sera with 35% \((\text{NH}_4)_2\text{SO}_4\), were covalently bound to the GRBC with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HC1 (Ott Chemical Co., Muskegon, Mich.). Conjugated GRBC and appropriate dilutions of either spleen, lymph node, or blood cells were added to 0.5 ml of 0.5% agarose (L'Industrie Biologique Francaise, Gennevilliers, France) at 45°C, and the suspension was immediately plated on microscope slides which were then incubated in a humidifying chamber for 1 h at 37°C. Plaque-forming cells (PFC) were developed by inverting the slides for 23/4 h in complement (1:10 dilution of guinea pig sera in BSS), either with amplifying antibody (sheep anti-rabbit IgG at an optimal dilution of 1:100) (indirect PFC), or without amplification (direct PFC). For differentiation of IgM- and IgG-PFC, slides were first immersed in 0.005 M dithiothreitol (DTT) (Calbiochem, San Diego,

1 Abbreviations used in this paper: BSS, balanced salt solution; DTT, dithiothreitol; GRBC, goat red blood cells; HGG, Cohn fraction II from human sera; IA, iodoacetamide; PFC, plaque-forming cells.
CYCLICAL APPEARANCE OF ANTIBODY-PRODUCING CELLS

Cyclical Appearance of Antibody-Producing Cells after a Single Injection of Antigen.—The splenic antibody-producing cell (PFC) response to a single intravenous injection of 2 mg aggregated HuIgG is shown in Fig. 1, which gives the average PFC from spleens of 5–20 rabbits on each day. PFC were first detected 3 days after injection and reached a peak on day 5. The number of PFC then decreased rapidly, with less than 20% of the peak number remaining on day 6. The number of PFC remained at a low level until day 12, at which time there was a significant increase. A second peak of PFC occurred on day 13, 8 days after the first peak, and was comparable to the level of the day 5 peak. 8 days later (21 days after injection) a third peak of PFC was detected, although not as high as the preceding peaks. Both direct and indirect PFC followed this pattern.

A similar type of response was seen in the spleen after a single intravenous injection of 2 mg aggregated TuIgG (Fig. 2). Indirect PFC reached a peak 4½ days after injection, decreased, and peaked a second time on day 12. In this case, the interval between peaks was again approximately 8 days. Direct PFC
Fig. 1. Kinetics of the appearance of PFC in spleens of rabbits after a single intravenous injection of 2 mg aggregated HuIgG.

Fig. 2. Kinetics of the appearance of PFC in spleens of rabbits after a single intravenous injection of 2 mg aggregated TuIgG.

were only 13% of the indirect PFC on day 4½ and were not detected on day 12, possibly because the plaque technique was not sensitive enough to detect the small number that would have been expected. On day 12, the indirect PFC were only 40% of the initial peak. The rapid disappearance of PFC after the day 5 peak was specific since the response to an injection of 2 mg of TuIgG was not affected by an injection of 2 mg of HuIgG given 4½ days previously.

**Correlation of Direct and Indirect PFC with 19S and 7S Antibody-Producing Cells.**—Reduction and alkylation of antibody produced by PFC (with DTT and IA, respectively) before addition of complement was used to determine the class of antibody to HuIgG secreted from spleen cells of rabbits injected with 2 mg of aggregated HuIgG. Direct PFC were inhibited at least 95% in both primary (day 5) and secondary responses (day 6), whereas indirect PFC were inhibited only 7% (Table I). Therefore, the sheep antirabbit IgG used as amplifying antibody would appear to be capable of both enhancing IgG PFC
and inhibiting IgM PFC. Treatment with IA alone had no significant effect on PFC.

*Secondary PFC Response.*—The splenic PFC response in primed rabbits (injected with 2 mg aggregated HuIgG 40–85 days before the second injection) represented a typical secondary response (Fig. 3). On day 2, the 1st day tested, there were already significant numbers of PFC, whereas PFC could not be detected until day 3 of the primary response. The peak PFC occurred on day 3 instead of day 5 (as shown in an experiment where indirect PFC in the peripheral blood on days 2, 3, and 4 after a second injection of antigen averaged 249, 3,923, and 452 PFC/10⁶ cells, respectively, whereas the primary response TABLE I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Primary response</th>
<th>Secondary response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Indirect</td>
</tr>
<tr>
<td>None</td>
<td>26</td>
<td>301</td>
</tr>
<tr>
<td>DTT + IA</td>
<td>0 (100%)</td>
<td>298 (0%)</td>
</tr>
<tr>
<td>IA</td>
<td>22 (14%)</td>
<td>303 (0%)</td>
</tr>
</tbody>
</table>

DTT = dithiothreitol; IA = iodoacetamide.

* Per 10⁶ spleen cells.

† The figures in parentheses represent the percent reduction in PFC of treated cells as compared with cells receiving no treatment.

Fig. 3. Kinetics of the appearance of PFC in spleens of rabbits after a second injection of 2 mg aggregated HuIgG. All rabbits were injected intravenously with a primary dose of 2 mg aggregated HuIgG 40–85 days before the second injection.
occurred on day 5 in both the spleen and blood). Also, the magnitude of the peak secondary response was approximately 10-fold greater than that of the primary response. No additional significant peaks of direct or indirect PFC were detected even though PFC were measured on day 6 and daily from days 8 to 16. Again, both indirect and direct PFC followed the same kinetics, although direct PFC were less than 20% of the indirect PFC. As each point represents the average PFC from only three rabbits, the minor variations in numbers of PFC that occurred were probably the result of animal variability.

Comparison of Spleen and Mesenteric Nodes.—Indirect PFC in the mesenteric lymph nodes were measured on days 5, 8, and 13 after a single intravenous injection of 2 mg aggregated HuIgG and compared with indirect PFC in the spleens of the same rabbits (Table II). The results, representing the average PFC per 10⁶ node or spleen cells of five rabbits for each day, show that PFC per 10⁶ cells in the nodes were approximately 50% of those in the spleen on day 5. By day 8, PFC in the nodes had decreased almost to background levels and, in contrast to splenic PFC, did not peak again on day 13.

Tissue Localization of HuIgG.—Rabbits were injected intravenously with 2 mg aggregated [¹²⁵I]HuIgG and exsanguinated on either day 2 or day 10. Lung, kidney, liver, spleen, mesenteric lymph node, and thymus (day 10 only) sections were taken for histological examination and for radioactivity measurements. Less than 5% of the injected radioactivity could be detected in the blood within 10 min after injection of the antigen. 2 days after injection, counts of sonicated tissues showed that lungs retained the greatest amount of TCA-precipitable radioactivity per gram of tissue (9.8 μg [¹²⁵I]HuIgG/g tissue) and spleens retained the next highest amount (1.14 μg [¹²⁵I]HuIgG/g tissue) (Table III). By day 10, the amount of antigen retained had decreased by approximately 90% in all tissues (giving a half-life of approximately 2.5 days) except the spleen. In contrast, the half-life of [¹²⁵I]HuIgG in the spleen was 12.8 days. Autoradiographs of spleen and lymph node sections on day 2 showed diffuse label throughout the follicles, whereas by day 10, label was concentrated in the germinal centers of the spleen, and no label could be detected in similar areas of the nodes (Fig. 4).

<table>
<thead>
<tr>
<th>Organ</th>
<th>Indirect PFC/10⁶ Cells</th>
<th>Day 5</th>
<th>Day 8</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>160.8</td>
<td>4.1</td>
<td>119.1</td>
<td></td>
</tr>
<tr>
<td>Nodes</td>
<td>75.5</td>
<td>9.0</td>
<td>11.5</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent average of five rabbits for each day.
Persistence of Immunogenicity of Injected HuIgG.—Irradiated rabbits injected with 2 mg aggregated HuIgG 24 h after irradiation (500 R) were injected with 1.8–2.1 $\times$ 10⁸ sensitized cells at various times after receiving the antigen. Cell suspensions were prepared from spleens and mesenteric lymph nodes of hyperimmune animals and injected (without antigen) in approximately equal proportions, spleen cells intravenously and node cells intravenously and intra-peritoneally. Spleens of the recipients were plaqued 3½ days after injection of hyperimmune cells. The number of indirect PFC was calculated as a percent.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Total TCA-ppt*</th>
<th>TCA-ppt*</th>
<th>Total TCA-ppt</th>
<th>TCA-ppt</th>
<th>$t_{1/2}$ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>10.62</td>
<td>9.8</td>
<td>0.91</td>
<td>0.86</td>
<td>2.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.63</td>
<td>0.26</td>
<td>0.03</td>
<td>0.02</td>
<td>2.5</td>
</tr>
<tr>
<td>Liver</td>
<td>0.25</td>
<td>0.15</td>
<td>0.02</td>
<td>0.02</td>
<td>2.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.36</td>
<td>1.14</td>
<td>0.79</td>
<td>0.74</td>
<td>12.8</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>0.17</td>
<td>0.09</td>
<td>0.01</td>
<td>0.01</td>
<td>2.5</td>
</tr>
<tr>
<td>Thymus</td>
<td>--</td>
<td>--</td>
<td>0.01</td>
<td>0.01</td>
<td>--</td>
</tr>
</tbody>
</table>

Values represent averages of three animals for each day.

* Trichloroacetic acid precipitable.

† The $t_{1/2}$ value was calculated between day 2 and day 10 and was based on TCA-precipitable activity.

Fig. 4. Autoradiographic evidence of follicular localization of $[^{125}\text{I}]$HuIgG in the spleens of rabbits stained with hematoxylin. (a) $\times$ 50; (b) $\times$ 320.
of indirect PFC in irradiated recipients receiving 2 mg aggregated HuIgG at the time of injection of sensitized cells. Rabbits receiving antigen 24 h after irradiation and not receiving sensitized cells showed essentially no PFC when plaqued at the same time. Based on the above criteria, 52% of the injected aggregated HuIgG remained in an immunogenic form in rabbits injected with antigen 10 days earlier (Fig. 5). By days 20 and 30, the effective concentration decreased to 12% and 3%, respectively. At days 60 and 90, essentially no "immunogenicity" remained.

**Effect of Dose of Antigen on Kinetics of PFC.**—Rabbits were injected with 0.02, 0.2, 2.0, or 20 mg aggregated HuIgG intravenously and spleens from each group were plaqued 4, 5, 6, 8, 12, 13, or 14 days after injection. With each dose of antigen, two peaks of PFC were detected (Fig. 6). In this single experiment,
the PFC to 2.0 mg aggregated HuIgG peaked on day 4 and the PFC to 0.02 mg aggregated HuIgG peaked day 6, while the PFC in rabbits injected with the other two doses peaked at the usual time, on day 5. Also, animals injected with 20 mg aggregated HuIgG showed a second peak later than day 13. The variability in time of appearance of peak PFC may reflect a true variability in the kinetic pattern among different rabbits. More likely, the variability is the result of the small number of animals used and the quantitative differences in PFC among different rabbits. In any event, with all doses, two peaks of PFC could be detected and were separated by approximately 8 days of markedly decreased responsiveness. The height of the second peak obtained with the various doses was inversely proportional to the height of the first peak and to the dose of antigen injected.

Kinetics of PFC in Spleen vs. Peripheral Blood.—The kinetics of the PFC response in the peripheral blood were essentially identical with that in the spleen as can be seen in Fig. 7, which shows the average PFC responses of four rabbits after injection of a single dose of 0.2 mg aggregated HuIgG. If there is a lag between appearance of PFC in the spleen and in the blood, it is less than 24 h as can be seen by comparing the PFC responses on days 12, 13, and 14.

Appearance of Precipitating Antibody in Relation to Appearance of PFC.—Spleens of rabbits were plaqued after intravenous injection of 2 mg aggregated HuIgG and sera from these rabbits analyzed for precipitating antibody. Precipitating antibody was too low to be detected until day 5 (the peak day for PFC) but rose to a peak on day 8 (Fig. 8). The antibody level decreased rapidly until after the second peak, when it rose again. When individual rabbits were analyzed (on days 5, 8, 13, 16, and 21 after injection of antigen) for PFC in their peripheral blood and precipitating antibody in their sera, the dependence of antibody on PFC can be seen clearly (Fig. 9). After the first peak of PFC on day 5 for rabbit 1, antibody peaked on day 8, then started to decrease until the second peak of PFC appeared on day 13. The serum antibody level then remained at a high level. In rabbit 2, no second peak of PFC occurred and antibody levels in the serum showed a continued decrease after peaking on day 8.
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Fig. 8. Relationship between PFC in spleens of rabbits and precipitating antibody in peripheral blood after a single injection of 2 mg aggregated HuIgG.

Fig. 9. Relationship between PFC and precipitating antibody in peripheral blood after a single intravenous injection of 2 mg aggregated HuIgG. The data are from representative rabbits which were bled 25 ml of blood (cardiac) on the respective days.

DISCUSSION

The above data suggest that a specific control mechanism regulates the formation of antibody-producing cells to a persisting antigen, resulting in the cyclical production of PFC. PFC in the spleens of rabbits were shown to appear, disappear, and reappear after a single intravenous injection of either aggregated HuIgG or aggregated TuIgG. After an initial peak of PFC on day 5, two subsequent peaks of PFC were observed at 8-day intervals, with a marked decrease in the number of PFC detected during these intervals. The levels of precipitating antibody in the serum reflected events occurring at the single cell level with a delay of approximately 3 days, accounting for the period of time needed for...
secretion and equilibration of antibody into the fluid spaces. Such fluctuations in PFC levels after a single injection of antigen have been described previously. Britton and Möller (5) showed cyclical peaks of PFC in mice injected intraperitoneally with heat-killed *Escherichia coli*. Sell et al. (13) demonstrated two peaks of IgA-γ1-, γ2 a-, and γ2 b-producing PFC in the spleens of mice after a single intravenous injection of SRBC. However, only a single peak of IgM-producing PFC was detected.

The identical kinetics of IgM and IgG synthesis in rabbits immunized with aggregated HuIgG indicates that if a switch from IgM to IgG production occurs, it takes place at a stage in differentiation before the secretion of immunoglobulins. Evidence for a switch has been observed by several groups of workers. Lymphocytes in rabbits have been shown to have cell surface IgM while synthesizing IgG, suggesting that after triggering of the cell by antigen there is a point when the cell switches from the synthesis of IgM to the synthesis of IgG (14). In the rabbit, this switch may explain why, for many antigens, the dominant molecular class for B-cell receptors is IgM, while in the plasma cell and serum IgG predominates (15). Pierce et al. (16) provided further evidence of a switch mechanism by inhibiting in vitro the IgG and IgA responses to SRBC with incorporation of anti-μ-chain into the cultures. Furthermore, the first appearance of IgG-PFC to SRBC in spleens of recipient mice injected with SRBC and normal lymphocytes was associated with clones of cells previously producing IgM (17-19). Other data are also available which indirectly support either an in vivo or in vitro switch in immunoglobulin class during differentiation (20-23). Direct evidence for double producers of IgG and IgM has been reported by Nossal and coworkers (24, 25), who were able to isolate cells containing both IgM and IgG. However, since only a few (1.5%) double producers were detected, it has been suggested that the majority of IgG-synthesizing cells could not have arisen from IgM producers (26-28).

An interesting aspect of the present study is that the appearance of PFC after a single intravenous injection of the antigen seems to be synchronous. Although it appears most likely that the apparent “synchrony” can be attributed at least indirectly to antibody, the precise mechanism is difficult to understand. It has often been demonstrated that passive antibody can interfere with the development of an immune response (cited in reference 1). Britton and Möller (5) have suggested that antibody controls fluctuations in the immune response to persisting antigens. In their experiments, a cyclical appearance of both antibody and PFC occurred after injection of the lipopolysaccharide of *Escherichia coli*, although synchrony was not
apparent. It has been proposed that the regulation of antibody production to both persisting and readily catabolizable antigens is controlled by antibody via a dynamic equilibrium among circulating antibody, antigen, and antigen-antibody complexes throughout the extracellular compartments (29). It would be difficult to explain the synchrony observed in the present experiments by such a mechanism since the interval between the peaks was not affected by the serum level of the antibody either produced or present at the time of appearance of the second PFC peak. However, the precise timing of peaks of PFC could be explained in part by antibody control if the regulating antibody was effective locally at the sites of production. The local concentration of antibody, in any given foci, would be the same in all animals, and it would be independent of the total amount in either the extra- or intravascular compartments. The latter possibility would explain the rapid decrease in PFC numbers which occurs regardless of the total antibody present in the serum. In addition, another event must occur to account for both the reappearance of PFC at a precise time and the longer induction period. Such synchrony could be explained by control mechanisms that have been described in other systems. The available data suggest that the regulation of cell renewal can be achieved by extending or contracting the length of the G₁ period (30, 31), and it appears that such regulation is tissue specific. In addition, cells from a variety of organs can be stimulated to begin dividing again after a period in which DNA synthesis had been arrested. From incorporation studies of cells blocked at different phases of the mitotic cycle, it has been determined that cells can enter mitosis upon an appropriate stimulus from G₁, or less likely, directly from G₂ (32). Furthermore, a latent period has been observed when cells can not respond to a stimulus by dividing (33). In spleens of rabbits producing PFC to aggregated HuIgG, inactivation of antigen by antibody produced locally could result in cells being arrested in some phase of the cell cycle. Only cells developed beyond a certain point would continue to differentiate to PFC, while cells developed to a lesser degree would be blocked temporarily from further division and differentiation. Upon termination of this latent period, cells would again be stimulated in the spleen by antigen uncovered when antibody diffused into the circulation. Thus, due to a transient curtailment of antigenic stimulation, cells that had not completed enough differentiation to become PFC without further antigenic stimulation may be arrested temporarily. When they are again capable of responding, antigen would be available in the spleen to restimulate them and synchronization of PFC could occur. In any event, it appears likely that the control of the latent periods and the synchrony of the appearance of PFC in the present system are exercised through some event(s) in the G₁ period. In this case, the effect on G₁ is probably the result of an indirect effect of antibody present at or near the site of its production.

The above reasoning would imply that although the initial peak of PFC results from the stimulation of virgin precursor cells, succeeding peaks must be the result of stimulation of memory cells. This same reasoning makes it difficult to imagine a mechanism for stimulation of virgin precursor cells over different periods of time that would result in such precise timing of peak PFC over a 1,000-fold range in the dose of antigen administered. Nor is it easy to visualize a mechanism that would result in the synchronized reappearance of
PFC arising directly from virgin B cells. Also, although the magnitude of the first peak of PFC varied directly with the dose of antigen administered, the second peak was inversely related to dose, suggesting that with large amounts of antigen, more cells were stimulated directly to PFC, leaving fewer memory cells available for restimulation. In contrast, with small amounts of antigen, cells were stimulated to differentiate, yet with most cells not enough hits per cell occurred to complete the pathway from precursor to PFC. This would explain why, after injection of extremely small amounts of antigen (0.02 mg), the second peak of PFC was greater than the first peak.

The cyclic appearance of peaks of PFC in the spleens of rabbits after an intravenous injection of aggregated HuIgG apparently depends on the persistence of antigen in this organ. The preferential persistence of this antigen in the spleen was demonstrated both functionally and directly by determining organ distribution of TCA-precipitable $[^{131}I]$HuIgG and by autoradiography. First, it was shown that sensitized cells could be specifically stimulated in irradiated recipients injected with aggregated HuIgG at least 30 days before transfer of the cells. Using similar cell transfer procedures, Britton et al. (34) have shown the persistence of SRBC for at least 14 days and *Escherichia coli* lipopolysaccharide for at least 45 days. Second, the injection of $[^{131}I]$HuIgG resulted in localization of some antigen in most of the tissues tested; however, the major localization was in the lung and spleen as would be expected after an intravenous injection. The labeled HuIgG was rapidly catabolized in the lungs and other tissues, but persisted in the spleen with a half-life of approximately 13 days. Autoradiography showed dense localization of the $[^{131}I]$HuIgG in the germinal centers of the spleen 10 days after injection, while no such localization was observed at this time in the lymph nodes. The failure to observe such localization in lymph nodes corresponds with the failure to observe a second peak of PFC in lymph nodes on day 13. Localization of antigen after various routes of injections has been extensively studied and was reviewed by Nossal and coworkers (35, 36). In the present studies, dense deposits of $[^{131}I]$HuIgG were localized in the central areas of germinal centers of spleens, but not in lymph nodes, 10 days after injection. It is not clear whether the dense localization in the germinal centers of the spleen was induced by antibody, as has been demonstrated with soluble antigens (35, 37), or whether after development of germinal centers and organ redistribution of antigen the aggregated antigen localized directly through the Fc fragment. Brown et al. (38) previously demonstrated that aggregated human gamma globulin, but not the monomeric form, rapidly localized in the germinal centers of the lymph nodes in a dendritic pattern after intradermal injection into guinea pigs. The failure of succeeding peaks in the secondary response may be related to the more rapid disappearance of antigen (39).

The PFC to HuIgG which appear in the peripheral blood apparently originate from cells stimulated in the spleen, as was proposed by Sorkin and coworkers (40, 41). The
identical kinetic pattern observed in cells from the blood and spleen are consistent
with an exit of cells from the spleen into the blood. Strober (42) has obtained evidence
for a qualitative change in the B cells after stimulation which allows them to migrate.
He observed that thoracic duct cells from primed donors were capable of responding
to antigen in an irradiated recipient after passage through an intermediate host,
while similarly passed normal thoracic duct cells were not able to respond, even though
capable of responding if not passed. The differences in the ability of the normal and
primed passed cells to respond were attributed to a change at the B-cell level, in
which the primed B2 cell became capable of recirculating, while the virgin B1 cell
could not. In the present situation, the ability of the stimulated B2 cell to enter the
circulation may be dependent on the loss of receptor sites during the process of differ-
entiation, thus freeing it from the antigenic stimulus. Depending on the degree of
stimulation, the B2 cell may either differentiate further into a PFC, or regenerate
receptors and return to the antigen deposits in the spleen (or other organs) where,
after a latent period, it can be stimulated to differentiate further. Retention of spec-
cifically sensitized cells from the circulation in lymphoid tissue containing the specific
antigen has been shown by Ford (43) and Durkin and Thorbecke (44). Migration of
stimulated cells has also been observed by Cannon and Wissler (45), who demonstr-
ated [H]thymidine-labeled cells in the blood of spleen-shielded, irradiated rats
after antigen stimulation. Unfortunately, the specificity of the cell in the blood was
not determined. Of course, not all cells stimulated by antigen and developing into B2
cells leave the spleen, since more PFC are usually found in the spleen than in the
peripheral blood. Other factors obviously play a role in whether a B2 cell leaves the
spleen or develops into either plasma or memory cells within the spleen, e.g., logistics
of the cell, effectiveness of antigenic stimulus, available space in the spleen, and poss-
ibly the inherent ability of each B2 cell to migrate.

A similar phenomenon has been observed in the cellular responsiveness of
rabbits to rabbit thyroglobulin after immunization with aqueous preparations
of bovine thyroglobulin (46). A major peak of PFC appeared in the spleen 25
days after a series of injections of bovine thyroglobulin was initiated and was
directed mainly against those determinants specific for bovine thyroglobulin.
7–8 days later, PFC in the thyroid gland reached a peak, and these, in contrast
to the early splenic PFC, were specific for only those determinants on bovine
thyroglobulin shared with rabbit thyroglobulin. Thus, it would appear that
rabbit thyroglobulin deposited in the injured gland was able to sequester B2
cells sensitized to determinants for rabbit thyroglobulin, and after an appro-
priate latent period possibly regulated by G1, stimulated the cells to produce
antibody to rabbit thyroglobulin.

SUMMARY

After a single intravenous injection of rabbits with aggregated HuIgG,
IgM- and IgG-plaque-forming cells (PFC) in both the spleens and peripheral
blood of rabbits peaked 5, 13, and 21 days after injection, while almost no PFC
could be detected on days 8 and 16. The available data suggest that the sec-
ondary peaks of PFC (days 13 and 21) resulted from stimulation of memory
cells by persisting antigen that was localized in the germinal centers in the spleen. No such persistence of antigen occurred in the lymph nodes, and these lymphoid tissues did not exhibit secondary peaks of PFC.

The identical kinetic patterns for IgM- and IgG-PFC indicate that the major portion of IgG-PFC did not result from IgM-secreting cells switching to IgG synthesis and secretion.

The present data suggest that the antibody produced and present at the site of interaction between committed cells and antigen is responsible for the regulation of antibody synthesis to persisting antigens. Possible cellular events involved in both the regulation and an apparent synchronous appearance of antibody producing cells in the spleens of rabbits were presented.

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