# Cytokinetics of IGG vs IGM PFU Response in the Lungs of C3H Mice[1] (34930)

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The hemolytic plaque assay procedure developed by Jerne (1) and by Ingraham and Bussard (2) has been used to evaluate the relative number of antibody-producing cells in the population. The number of plaque-forming cells detected during the primary and secondary responses is related to the levels of antibody titers attained in the circulation which in turn determines the immune status of the animal. The contribution of antibodies to the circulation by antibody-forming cells residing in extralymphoidal tissues is not known. The possibility that significant amounts may come from this source cannot be ignored.

If, in the course of preliminary stimulation, immunocompetent cells seed or are stimulated *in situ* in nonlymphoid organs, activation of these cells and their progeny by secondary stimulation will indicate their whereabouts.

These studies were initiated to determine the presence of antibody-forming cells in the lung of hyperimmunized mice.

*Materials and Methods.* $C_3H$ strain mice were injected with 0.1 ml of a 50% suspension of sheep erythrocytes (SRBC) and sacrificed at periodic intervals. The spleens from such animals were tested *in vitro* as follows: single cell suspensions were obtained by teasing with a 23-gauge needle in Hanks' solution. One-tenth milliliter of the suspension was mixed with 3 ml of 0.8% agar containing 1 mg diethylaminoethyl dextran. To this mixture 0.2 ml of a 14% SRBC suspension was added, and the mixture was plated in petri dishes containing 10 ml of 1.4% agar. All plates were incubated at 37° for 2 hr and then layered with 3 ml of complement. Incubation was continued for 1 hr at 37°, the plates were decanted, washed with saline, and stained with benzidine.

The peripheral blood cells were isolated according to the method of Möller (3) with slight modification. Peripheral blood cells were obtained by mixing 1.0–1.5 ml of blood with 2.0 ml heparinized saline containing 3% dextran 250. The tubes were stoppered, and the red cells were allowed to settle at room temperature until the supernatant fluid was relatively free of erythrocytes. The supernatant fluid containing the lymphoid elements was diluted in Hanks' solution and centrifuged at 500 rpm. The twice-washed cells were plated to determine the number of plaque-forming units (pfu).

Lung tissue was teased apart in cold Hanks' solution with a needle and forcep and passed through a U.S. Standard Sieve No. 200 screen. The cells were washed and suspended to the desired concentration and plated as above. Due to the spongy and elastic nature of lung tissue, it was extremely difficult to tease the entire organ apart, and, although single cells could be obtained, cell counts generally showed the numbers to be relatively low.

Detection of 7S pfu was made by incorporating 0.1 ml optimally diluted rabbit anti-mouse globulin serum into the plating medium. A distinction between 19S and 7S pfu was made comparing agar plates containing rabbit antimouse globulin with agar plates containing no rabbit anti-mouse globulin. The increase in pfu detected by the plates with the rabbit antibody over the number

[1] This investigation was supported by the National Institute of Allergy and Infectious Diseases Grant AI 798501 and the National Science Foundation Grant GB 6860.

TABLE I. Rise and Fall of Plaque-Forming Cells in the Spleen, Peripheral Blood, and Lungs of $C_3H$ Mice After Primary Intra-abdominal Injection of Sheep Red Blood Cells.

| Time after primary antigen injection (days) | No. of animals | Mean number of pfu/$10^6$ cells plated | | | | | |
|---|---|---|---|---|---|---|---|
| | | Spleen | | Peripheral blood | | Lungs | |
| | | 19S | 7S[a] | 19S | 7S[a] | 19S | 7S[a] |
| 3 | 3 | 21 | <1 | 12 | 0 | 0 | 0 |
| 4 | 28 | 176 | 54 | 288 | 32 | 3 | <1 |
| 5 | 9 | 71 | 89 | <1 | <1 | <1 | <1 |
| 6 | 13 | 24 | 148 | 0 | <1 | 1 | <1 |
| 7 | 4 | 8 | 57 | 0 | 0 | 0 | <1 |
| 8 | 4 | 5 | 30 | 1 | 0 | 0 | 0 |

[a] Rabbit anti-mouse globulin was incorporated into the agar medium at the time of plating.

obtained by the plates without the rabbit antibody was considered 7S pfu.

Cell counts were made on samples after the red blood cells were lysed with 0.1 *M* HCl.

*Results.* In the first series, $C_3H$ mice were injected with SRBC on Day 0 and the assay for pfu was initiated on Day 3 and continued to Day 8. The spleen, peripheral blood cells, and lung cells were plated. As seen in Table I, the number of 19S pfu per $10^6$ spleen cells peaked at Day 4 and continued to decrease thereafter. In these animals, there was a perceptible increase in 7S pfu by Day 5; this response peaked on Day 6 and continued to decrease until Day 8.

The peripheral blood showed presence of 19S pfu on Day 3 with a sharp increase on Day 4. By Day 5 essentially no 19S pfu were detected in the circulation. The reason for this transient appearance and quick disappearance is not known. The 7S pfu response, if any, was seen only on Day 4.

The lung showed only 3 pfu/$10^6$ cells plated on Day 3. This was probably due to 19S pfu from the circulation in transient passage through the lungs. No significant numbers of 7S pfu were detected in the lungs during this period.

Since peak levels of 19S pfu are released to the circulation on Day 4, progenitor cells as well as presensitized immunocompetent cells may also be distributed in the various nonlymphoid organs. If such presensitized cells are present in the lungs, activation of these cells and their progeny may be possible after specific antigen stimulation.

In the second series of experiments animals were given a primary injection on Day 0 and a second antigenic stimulation 21 days after the primary inoculation. Varying numbers of animals were sacrificed from Day 22 to Day 29 and the spleen, peripheral blood, and lung tissues assayed for the presence of both 19S and 7S pfu.

The spleen showed a slight rise in 19S pfu on Days 24 and 25, approximately 1500 pfu/total spleen (Table II). The 7S pfu showed a steady increase from Day 22 to Day 26. The peak response overlapped two days (*i.e.*, Day 4 and Day 5 after the last injection). Although the response continued to decline, thereafter there were still significant numbers of 7S pfu detectable in the spleen on Day 29.

Surprisingly, during this entire period no significant numbers of 7S pfu were seen in the peripheral blood of these mice.

The lung, however, showed a perceptible rise in 7S pfu on Day 25 (4 days after the last injection) and the number of pfu increased until Day 27. By Day 29 less than one 7S pfu/$10^6$ cells was present. No significant levels of 19S pfu were seen in the lungs during this interval. A composite plot of primary and secondary responses of the various tissues plated is given in Fig. 1. It is interesting to note that in the animals given one injection the 7S pfu response apparently begins on Day 4 and overlaps the 19S pfu response. The 19S peripheral blood pfu peaks on Day 4 but the sharp drop-off by Day 5 occurs during the period when the 7S pfu

TABLE II. Rise and Fall of Plaque-Forming Cells in the Spleen, Peripheral Blood, and Lungs of $C_3H$ Mice After Secondary Intra-abdominal Injection of Sheep Red Blood Cells Was Given 21 Days After the Primary.

| Time after primary antigen injection (days) | No. of animals | Mean number of pfu/$10^6$ cells plated | | | | | |
|---|---|---|---|---|---|---|---|
| | | Spleen | | Peripheral blood | | Lungs | |
| | | 19S | 7S[a] | 19S | 7S[a] | 19S | 7S[a] |
| 22 | 2 | <1 | 4 | 0 | 0 | 0 | 0 |
| 23 | 2 | 3 | 21 | 1 | 0 | 0 | 1 |
| 24 | 2 | 8 | 108 | 0 | 0 | 0 | 0 |
| 25 | 7 | 7 | 521 | 0 | 0 | <1 | 47 |
| 26 | 4 | 4 | 528 | 0 | 0 | 0 | 88 |
| 27 | 4 | 2 | 365 | <1 | 0 | 0 | 292 |
| 28 | 4 | 2 | 291 | 0 | 0 | 0 | 43 |
| 29 | 5 | 3 | 186 | <1 | 0 | <1 | <1 |

[a] Rabbit anti-mouse globulin was incorporated into the agar medium at the time of plating.

level is rising in the spleen.

In contrast, the secondary stimulation caused only a slight 19S spleen pfu response; however, an exceedingly intense 7S response occurred during this period. No 7S pfu were seen in the blood but a noticeable rise in lung 7S pfu can be seen. The appearance of these pfu in the lungs cannot be reasonably attributed to spleen 7S pfu seeding by way of the circulation. Therefore, we are inclined to believe that presensitized cells were localized after primary immunization and stimulated *in situ* in the organ by the second antigen injection.

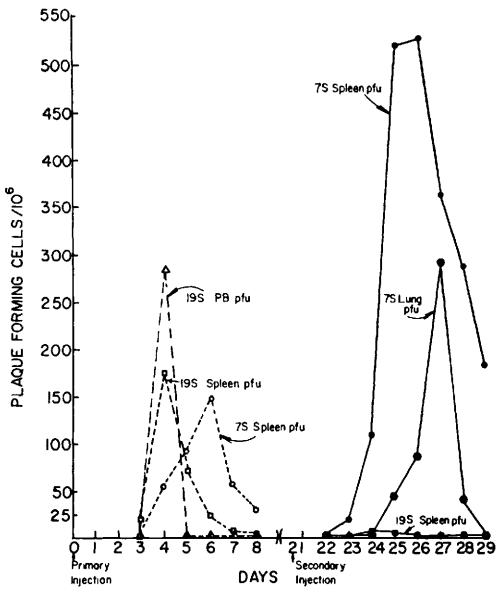


FIG. 1. A composite plot of primary and secondary responses seen in the spleen, peripheral blood (PB), and lungs of $C_3H$ mice injected with sheep red blood cells.

*Discussion.* It is evident that the cells of the spleen and lymph nodes are involved in the cellular antibody production during the first 4–5 days after antigen administration. Such cells can also be found in the circulating blood (4–6). However, this response is very transient (7). The destination of the antibody-forming cells in the blood is of particular interest because they may seed the extralymphoidal organs. Marshall and White (8) in earlier studies showed that when animals are given repeated injections of antigen, plasma cells appear in the lungs, liver, bone marrow, and kidneys. The largest number of cells was present in the lungs. Although no direct method was used to detect antibody in these plasma cells, the rise in the serum titers at the time these cells appeared indicated that the cells may be involved in 7S and globulin synthesis.

The results reported here show that after a primary injection of SRBC there was a rise in the 19S pfu in the spleen which peaked on Day 4. At this time there was a sharp but transient rise of the 19S pfu in the blood.

By applying the indirect technique for plating, it appears that the initial response is mainly of the IgM type. The first indication of 7S pfu using the indirect method occurred

5 days after the primary injection of antigen. During this period there was a substantial decrease in the 19S pfu of the spleen. This decrease of 19S pfu is presumed to be the result of 7S antibody synthesis causing a feedback inhibition. This effect was previously described by Uhr *et al.* (9) and Möller and Wigzell (10). We believe the quick disappearance of 19S pfu from the blood is due to the same mechanism, *i.e.*, increased levels of 7S antibody in the circulation at this time. During this period there was a negligible level of 19S pfu in the lung.

Animals primed with antigen and challenged 21 days later gave a somewhat different response. There was an intense 7S pfu response and a low 19S pfu response over this period. Essentially no 19S or 7S were detected in the peripheral circulation. A perceptible rise in the 7S pfu was seen in the lungs on Day 4 after the second injection. This response peaked on Day 6 and had disappeared by Day 8. No appreciable 19S pfu was seen in the lungs over this period.

Although it was a simple matter to prepare cells from the spleen for plating, it was difficult to do so from the lungs. The number of lymphoid cells in the lungs is not known. The percentage of these cells recovered and the biologic activity of the preparation would influence the plating results.

These studies show that the lungs appear to be prime sites for localization of antibody-forming cells. We believe that such cells arrive primarily from the lymphoid organs where the initial events of antibody synthesis begin. Presumably, these migratory precursor cells pass by way of the circulation and seed the various organs such as kidneys, liver, adrenal glands, and under secondary stimulation actively divide and synthesize antibodies. An alternative view which the data does not exclude is that cells in the lungs or other organs may be primed by an initial exposure to antigen, but only produce detectable antibody after secondary stimulation.

Immunofluorescence studies using guinea pigs hyperimmunized to egg albumin and human gamma globulin antigens indicate that the antibody-containing plasma cells in the lungs could be seen in the stroma surrounding pulmonary vessels and also in the alveolar walls. Occasionally cells were found within the alveoli (11). Presently it is not possible to estimate the total number of antibody-forming cells in the lungs; however, significant numbers appear to be involved during the secondary response of the host.

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Received Feb. 24, 1970. P.S.E.B.M., 1970, Vol. 134.