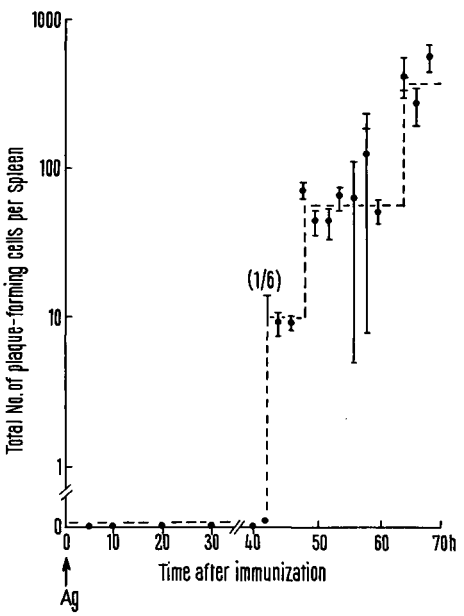
Rapid Recruitment and Proliferation of Antibody Plague-Forming Cells in the Absence of Natural Antibody

Induction of antibody formation is directly related to cellular events, including differentiation, proliferation, and recruitment of cells present in the lymphoid system. Recently much attention has been directed towards study of the kinetics of appearance of specific antibody producing cells[1-6]. However, the earliest events of the immune response, probably the most informative phase of immunogenesis, is still poorly understood. This is due mainly to the fact that all available model systems which permit direct detection and enumeration of individual antibody-secreting cells also reveal the presence of a 'background' of pre-existing or 'natural' antibody-producing cells[7-14]. In this regard, we have recently described a new model system based on the Pfeiffer phenomenon of vibriolysis to enumerate specific immunocytes in lymphoid organs of mice immunized with *Vibrio cholerae*. To our surprise, no antibody forming cells to this micro-organism could be detected in normal, non-immunized mice. The availability of this very sensitive system without a 'background' of natural antibody activity prior to immunization offered a unique opportunity to study the earliest cellular events during immunogenesis.

The vibriolytic immunoplaque assay was utilized to determine precisely the time of appearance of the first recognizable antibody plaque forming cells (PFC), at closely spaced time intervals after immunization, using whole spleen cell populations. For this purpose groups of NIH albino A mice were immunized i.v. with 5 μg of a heat-killed vaccine of *V. cholerae*[15-18]. 6 or more mice were killed at various times after immunization and their spleens assayed for PFCs by the direct vibriolytic plaque assay, exactly as described previously[16].

The critical period of PFC differentiation was determined by pilot experiments. Between 12 and 40 h after immunization, not a single PFC was detectable in the entire spleen of any test animal. Groups of 6–15 animals were then killed and tested at 2 h intervals between 40 and 72 h of the response (Figure). One of 6 animals tested 42 h after immunization had a few PFCs in the spleen; the other mice were negative. At 44 h, 5 of 6 spleens tested had PFCs, with an average of 9 per spleen. Thus, it could be concluded that the first antibody forming cells appear after a consistent latent period of 42–46 h, with an average number of 9–10 PFC per spleen. At 48 h, there was an abrupt increase to 50–60 PFCs per spleen (Figure). This number remained constant for 12 h until a third increase in the number of PFCs occurred at 62 h. Thus, there was a 'stepwise' pattern of PFC accumulation in the spleen between 42 and 72 h after immunization.



Number of plaque forming cells in whole spleen (average of 6–15 mice ± S.E.) at close intervals after single i.v. immunization (Ag) with 5 μg of *V. cholerae* strain Ogawa vaccine (Only 1 of 6 animals positive at 42 h). Ag, day of antigen injection.

No detectable serum antibody to vibrio appeared until at least 4 days after immunization[15,16] and indirect PFCs (presumably IgG antibody producers) facilitable

Suppressive effect of cytosinarbinoside on appearance of vibriolytic antibody forming cells in spleens of mice treated at various times during first few days after immunization

Time of drug injection (h after immunization)[a]	Number of PFC per spleen[b] 48 h	96 h
	Normal control mice	
–	60	2972
	Drug treated mice	
6, 12, 24	34	–
24, 30, 36	2	–
48, 64, 72, 80	–	0

[a] Groups of mice injected i.p. with 100 μg of drug as indicated. [b] Average of the number of PFCs in 6–8 spleens tested at time indicated after i.v. immunization with 5 μg vaccine *V. cholerae* Ogawa.

[1] N. K. Jerne, A. A. Nordin and C. Henry, in *Cell Bound Antibodies* (Eds. H. Ames and H. Koprowski; Wistar Institute Press, Philadelphia 1963), p. 109.
[2] H. Wigzell, G. Moller and B. Andersson, Acta path. Microbiol. scand. *66*, 530 (1966).
[3] J. S. Hege and L. J. Cole, J. Immun. *96*, 559 (1966).
[4] W. J. K. Tannenberg and A. N. Malaviya, J. exp. Med. *128*, 895 (1968).
[5] D. A. Rowley, F. W. Fitch, D. E. Mosier, S. Solliday, L. W. Coppleson and B. W. Brown, J. exp. Med. *127*, 983 (1968).
[6] T. Makinodan, T. Sado, D. L. Groves and G. Price, in Curr. Topics Microbiol. Immunol. *49*, 30 (1969).
[7] J. Sterzl, J. Vesely, M. Jilek and L. Mandel, in *Molecular Cellular Basis of Antibody Formation* (Ed. J. Sterzl; Czech. Academy of Science, Prague 1965), p. 463.
[8] V. Cheng and J. J. Trentin, Proc. Soc. exp. Biol. Med. *126*, 467 (1967).
[9] S. Hirano and H. Friedman, Nature, Lond. *224*, 1316 (1969).
[10] M. Landy, R. P. Sanderson and A. L. Jackson, J. exp. Med. *122*, 483 (1965).
[11] S. A. Schwartz and W. Braun, Science *149*, 200 (1965).
[12] B. J. Baker, H. Bernstein, V. Pasanen and M. Landy, J. Immun. *97*, 767 (1966).
[13] G. M. Shearer and G. Cudkowicz, J. Immun. *101*, 1263 (1968).
[14] G. Biozzi, C. Stiffel, D. Monton, Y. Bouthillier and C. Decreusefond, Immunology *14*, 7 (1968).
[15] R. F. McAlack, J. L. Allen and H. Friedman, J. reticuloendoth. Soc. *5*, 3 (1968).
[16] R. F. McAlak, J. Cerny, J. L. Allen and H. Friedman, Science *168*, 141 (1970).
[17] J. Cerny, R. F. McAlak and H. Friedman, Fedn Proc. *29*, 769 (1970).
[18] R. F. McAlak, J. Cerny, M. Sajid and H. Friedman, Bact. Proc. *21*, 111 (1970).

by heterologous anti-globulin serum appeared only after the 7th day[18]. Thus, the first PFCs appear to represent a homogenous population of IgM producing cells and circulating antibody probably has no direct regulatory role in their stepwise appearance. It seemed likely that the initial appearance of PFCs was due to a non-random process of recruitment of PFCs from a pool of as yet uncharacterized precursors.

To test the importance of cellular proliferation in this response cytosine-β-D-arabinofuranoside HCL (CA) was used as a specific inhibitor of DNA synthesis[19,20]. For this purpose CA was injected repeatedly during the 1st, 2nd or 3rd day after immunization into parallel groups of mice. The number of PFCs per spleen was determined at 48 and 96 h (Table). When CA was injected between 6 and 24 h after immunization there was only a moderate depression in the number of PFC detected at 48 h. However, when CA was injected between 24 and 36 h the number of PFCs detected at 48 h was almost completely suppressed. Injections of CA between 48 and 72 h also completely suppressed PFC formation at 96 h (Table).

Thus, it seems reasonable to conclude that an antigen induced DNA synthesis and proliferation of cells occurs mainly during the second day of the latent period and that this proliferation is essential for both specific differentiation of antibody forming cells and their accumulation. On the other hand, the nature of this proliferation is by no means clear. It seems highly unlikely that the first PFCs arise by a symmetric division of some hypothetical precursor cells during the latent period, since even 1 such precursor cell, with a generation time of 5–7 h[21–24], would give a higher number of PFC progeny than that found experimentally. Furthermore, the 'staircase' type of cell accumulation to sheep erythrocytes described recently by others[23] also would not reflect a simple clonal 'growth' of PFCs. Therefore, the results of this study support the concept that antibody forming cell differentiation is the result of more complicated cellular processes, including cell interaction[24–26] and recruitment from a compartment of proliferating precursors[1–3,21–23].

To overcome the difficulties due to natural immune background, STERZL et al.[27] and KIM and WATSON[28] have resorted to the use of germ-free precolostral piglets to study the earliest phase of differentiation of hemolytic PFCs to sheep red blood cells. Similar to the results described in this study with conventionally bred mice lacking a pre-existing background to cholera bacilli, they also found a definite latent period of approximately 2 days before first appearance of hemolytic PFCs. Thereafter there was a rapid increase in the number of PFCs, generally greater than that which could be accounted for by simple cellular proliferation. The consistency of the latent period of immune response to 2 unrelated antigens in experimental systems without a natural immune background suggests that a specified time period may be necessary for certain cellular events initiated by antigen stimulation before the first antibody can be synthesized or released[29].

Zusammenfassung. Nachweis, dass «Plaque»-bildende Antikörperzellen gegen somatische Antigene von *Vibrio cholerae* sich in der Milz immunisierter Mäuse schnell und «treppenartig» nach übereinstimmender Latenzzeit von 42 h entwickeln, ohne dass dabei ein «background» an Antikörper bildenden Zellen zu erfassen war.

J. CERNY, R. F. MCALACK and H. FRIEDMAN

Departments of Microbiology, Albert Einstein Medical Center and Temple University Medical School, Philadelphia (Pennsylvania 19141, USA), 13 November 1970.

[19] M. Y. CHU and G. A. FISCHER, Biochem. Pharmac. *11*, 423 (1962).
[20] J. A. STECHK, in *Experimental Chemotherapy* (Eds. R. J. SCHNITZER and F. HAWKING; Academic Press, New York 1966), vol. IV, p. 79.
[21] R. W. DUTTON and R. I. MISHELL, Cold Spring Harbor Symp. quant. Biol. *32*, 407 (1967).
[22] E. E. CAPALBO and T. MAKINODAN, J. Immunol. *92*, 234 (1964).
[23] E. H. PERKINS, T. SADO and T. MANKINODAN, J. Immun. *103*, 668 (1969).
[24] H. N. CLAMAN, E. A. CHAPPERON and R. F. TRIPLETT, Proc. Soc. exp. Biol. Med. *122*, 1167 (1966).
[25] J. F. A. P. MILLER and G. F. MITCHELL, J. exp. Med. *128*, 801 (1968).
[26] D. E. MOSIER and L. W. COPPLESON, Proc. natn. Acad. Sci., USA *61*, 542 (1968).
[27] J. STERZL, Cold Spring Harb. Symp. quant. Biol. *32*, 493 (1967).
[28] Y. B. KIM and D. W. WATSON, Fedn Proc. *27*, 493 (1968).
[29] Supported, in part, by research grants from the U.S. National Science Foundation and the National Institutes of Health. We thank Mrs. JACKIE FRONTON and Mrs. HOMA KAMALI for excellent technical assistance.

Bile Salts and Platelet Aggregation

Relatively large amounts of taurine are known to be present in human platelets[1]. Since taurine is present in bile conjugated with bile salts, it seemed to us not unreasonable to consider the heretofore unsuspected possibility that some platelet taurine might be conjugated with bile salts which might play a role in platelet aggregation. It it not known, however, whether bile salts influence the aggregation of platelets. We report here on the effect of bile salts on platelet aggregation and plug formation in human citrated platelet-rich plasma (PRP).

Platelet aggregation and plug formation was studied in the rotating loop system as previously described[2] except that the blood was collected into 2.69% trisodium citrate. All bile salts were purchased from Calbiochem, Los Angeles, California, and were labelled Grade A. In 15 experiments (9 different plasmas) it was observed that rapid platelet aggregation and plug formation always occurred in response to sodium deoxycholate (DOC) at a final concentration of 1.7 mM and in several cases to as little as 0.43 mM. Several taurine conjugates of bile salts were also studied and found to cause platelet aggregation. All were less active than DOC. Their activity in descending order was: sodium taurodeoxycholate > sodium taurochenodeoxycholate > sodium taurocholate (Table). Free taurine had little or no influence on platelet aggregation (Table).

During the course of these studies it was noted that at a fixed concentration of DOC the aggregation parameters improved as the interval between obtaining the blood sample and performing the test was increased. Qualitatively similar results were obtained with sodium taurodeoxycholate and sodium taurochenodeoxycholate.