single limiting membrane. The particles were rather uniform in size approximately 400 Å in diameter and were composed of a thick electron-dense outer wall and an electron-lucent center. Less than 1% of the particles contained small nucleoids which resembled the A-type particles as described by BERNHARD[4].

In some cytoplasmic inclusions, whorled membranes were formed (Figure 3). Particles lying between them were occassionally seen. Some inclusions consisted entirely of whorled membranes (Figure 2). In a few instances, budding of virus-like particles from the membranes was suggested.

The origin of the membrane-bounded virus containing inclusions may possibly be from mitochondria. Some inclusions have the size as mitochondria, some inclusions have only a few whorled membranes with a few particles (Figure 3). These may be suggested as transitional forms between the mitochondria and virus containing inclusions[5].

Zusammenfassung. In Knochenmark-Monocyten eines fünfjährigen Mädchens konnten im Cytoplasma Einschlüsse beobachtet werden, die mit gewundenen Membranen und virusähnlichen Partikeln gefüllt waren.

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2 May 1972.

[4] W. BERNHARD, Cancer Res. *18*, 18491 (1958).
[5] Technical assistance of J. MEADOR is greatly appreciated.

Antigen Binding Rosette Forming Cells in a Friend Virus-Induced Leukemia

The suppression of immunological functions induced by infection of experimental animals with RNA tumorigenic viruses is a poorly understood phenomenon[1]. Infection with a virus before administration of antigen has a greater suppressive effect of the subsequent antibody response than does infection after immunization[2,3]. Furthermore, spleen cells from a virus-infected donor show decreased immunological potential when transferred adoptively to an immunoincompetent recipient[4]. These findings imply that the leukemogenic virus causes a defect at the level of immunologically competent antigen reactive cells (ARC). Does the virus eliminate the ARCs by destroying them, or do the ARCs remain preserved in a state of functional paralysis prevented from differentiating into antibody production upon antigenic stimulation? The following experiments were undertaken in an attempt to distinguish between the two aforementioned alternative mechanisms of viral inhibition of the antibody response. The effect of Friend leukemia virus (FLV) on rosette forming cells, which are capable of binding antigen (heterologous erythrocytes) in vitro, was chosen as a model system.

Six- to twelve-week-old male Balb/c mice (Flow Laboratories, Rockville, Md.) were used. Friend leukemia virus was obtained from Dr. W. CEGLOWSKI, The Pennsylvania State University[3,4].

Rosette forming cells (RFC) in mouse spleen were enumerated by incubation of a spleen cell suspension with sheep red blood cells (sheep RBC) according to the technique of BIOZZI et al.[5], with two modifications: a) buffered Hanks' balanced salt solution was used for cell suspension instead of EDTA buffer and b) cells were

Table I. Effect of previous FLV infection[a] on the response of specific plaque forming cells (PFC) and rosette forming cells (RFC) to immunization with 10^8 sheep RBC

Interval[b]	PFC		RFC/10^6 cells
(days)	per spleen	per 10^6 cells[c]	
1	66700 (± 10950)[d]	420 (± 91)	38000 (± 26400)
Control	66600 (± 26200)	426 (± 132)	24000 (± 7600)
5[e]	7700 (± 2100)	39 (± 10)	8400 (± 3300)
Control[e]	68600 (± 27370)	430 (± 40)	26400 (± 1000)

Immune responses were assayed in the spleen 4 days after immunization. [a] Friend leukemia virus (FLV)[3] was prepared as a 10% (w/v) homogenate of spleens from infected Balb/c mice, in Puck's saline (Gibco, N.Y.), pH 7.4. The homogenate was subjected to 2 centrifugations (1,500 *g*, 15 min, 4 °C and at 7,000 *g*, 10 min, 4 °C, respectively). The final supernatant fraction was filtered through a Millipore filter (0.8 μm) and frozen at −80 °C. Activity of the virus was determined by the spleen colony assay[14]. Each mouse received i.v. 5,000–10,000 focus forming units of FLV. [b] Interval in days between infection with FLV and immunization with SRBC. Controls were uninfected mice of the same age. [c] Calculated on the basis of approximately 10^6 cells in 0.1 ml of 1% (v/v) spleen cells suspension. [d] Arithmetic mean from 5–10 individual mice (± standard deviation). [e] Difference between infected and control animals in both PFC and RFC responses, respectively, is significant ($p < 0.01$) as calculated by a rank test[15].

Table II. Number of background plaque forming cells (PFC) and rosette forming cells (RFC) in spleens of non-immunized, FLV-infected mice

Interval after infection (days)[a]	PFC per spleen	per 10^6 cells[b]	RFC/10^6 cells
3			820 (± 53)[c]
Control			1040 (± 258)
5	277 (± 260)[d]	2 (± 1.3)	1700 (± 245)
Control	28 (± 28)[d]	<1[e]	1000 (± 447)

[a] For preparation and infection with FLV see Table 1; controls were uninfected mice of the same age. [b] Calculated on the basis of approximately 10^6 cells in 0.1 ml of 1% (v/v) spleen cell suspension. [c] Arithmetic mean from 6 individual mice (± s.d.). [d] Statistically significant difference between infected and control group ($p < 0.01$) by the rank test. [e] 1 to 5 PFC detected in 0.1 ml of 10% suspension (10^7 cells).

[1] A. L. NOTKINS, S. E. MERGENHAGEN and R. J. HOWARD, A. Rev. Microbiol. *24*, 522 (1970).
[2] M. H. SALAMAN and N. WEDDERBURN, Immunology *10*, 445 (1966).
[3] W. S. CEGLOWSKI and H. FRIEDMAN, J. Immun. *101*, 594 (1968).
[4] W. S. CEGLOWSKI and H. FRIEDMAN, J. Immun. *105*, 1406 (1970).
[5] G. BIOZZI, R. A. BINAGHI, C. STIFFEL and D. MOUTON, Immunology *16*, 349 (1969).

incubated for 3 h at 4 °C. Antibody plaque-forming cells (PFC) against sheep RBC were enumerated in aliquots of the same spleen cell suspension by the agar plaque technique[6].

The immunosuppressive effect of Friend leukemia virus (FLV) increases with the interval between virus infection and subsequent immunization with RBC (Table I). Infection with the virus 5 days before immunization with sheep RBC resulted in marked inhibition of the immune response to erythrocytes; both the number of hemolytic PFC and the number of newly arrising rosette forming cells (RFC) in the spleen were suppressed when compared to the response of uninfected controls.

In contrast, spleens of non-immunized mice treated with the virus contained as many antigen binding RFC as spleens of normal mice (Table II). We noticed a significant enhancement of background PFC by the virus infection even without administration of sheep RBC (Table II). This finding correlated with the earlier observation of others[7]. There is no explanation of this phenomenon presently available but preliminary results of cell tranfer experiments indicate that the increase of background PFC is due to the effect of virus upon already existing antibody forming cells rather than the formation of new PCF from precursor cells (Cerny, unpublished results).

The finding of normal numbers of RFC in FLV-infected spleen accompanied by relative unresponsiveness to immunization with sheep RBC raised a question about the nature of erythrocyte binding on mouse spleen cells. It seemed possible that the virus infection, followed by a cell transformation, could produce a structural change on the surface of splenocytes resulting in a non-specific adhesion of sheep RBC. To test this possibility spleen cell suspensions were prepared from the following groups of mice: a) mice immunized with sheep RBC; b) mice infected with FLV and immunized with sheep RBC; c) mice infected with FLV. Pretreatment of cells with heterologous anti-mouse globulin serum specifically and equally suppressed formation of rosettes in each of the 3 cell suspensions (Table III). Thus it appears that the binding of sheep RBC on both normal and FLV-infected mouse spleen cells is mediated by an immunologlobulin on the cells, and is a specific phenomenon.

Results of experiments in several laboratories have shown that selective removal of rosette-forming cells[8] or of antigen binding cells[9] resulted in specific diminution of immunological competence of the remaining lymphoid cells against that antigen. Furthermore, it was demonstrated previously (as well as in this report) that antigen binding on the cell surface is mediated by an immunoglobulin receptor[5,10]. Thus it can be postulated that the natural rosette forming cells represent at least a fraction of antigen-reactive cells. On the other hand, it is likely that the increasing number of RFC after immunization with erythrocytes represents a mixture of newly generated antibody secreting cells and non-secreting antibody carrying cells[10].

Data presented here (i.e., no inhibition by FLV of rosette formation, but inhibition of the immune response to sheep RBC) suggest that antigen reactive cells may remain preserved during a virus induced leukemogenesis, whereas differentiation leading to antibody production is inhibited. This possibility is further strengthened by our finding that a spontaneous regression of virus induced leukemia – observed in some experimental systems – is followed by a rapid recovery of immunological responsiveness and even by hyperreactivity to an antigenic challenge[11]. Thus the virus induced immunosuppression appears to be analogous to the phenomenon of antigen induced immunosuppression – immunological tolerance. Lympoid tissues of an immunologically tolerant rodent contain normal numbers of antigen binding cells[12] and the period of unresponsiveness is sometimes followed by hyperreactivity[13]. Studies on the relationship between tolerance and virus induced immunosuppression may reveal an essential control mechanism in immunological cellular differentiation[16].

Résumé. Les souris infectées par le virus de la leucémie de Friend avaient le meme nombre de cellules spléniques capables de se lier à des érythrocytes de mouton grâce à un récepteur immunolgobuline que les souris normales, non infectées, et servant de contrôle, possédent également. De plus, les souris infectées par le virus répondent à l'immunisation par des érythrocytes de mouton defacon nettement diminuée.

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Table III. Inhibition of splenic rosette forming cells (RFC) with anti-globulin serum[a]

Origin of spleen cells[b]	RFC per 10^6 spleen cells	
	RaM[c]	NRS[d]
A) SRBC immune	<100[e]	52,000
B) FLV-infected SRBC-immune	<100	26,000
C) FLV-infected	<100	2,500

[a] Sera used for inhibition were rabbit anti-mouse immunoglobulin polyvalent serum (RaM) (Microbiological Associated, Bethesda, Md.) and normal rabbit serum (NRS) (Difco, Michigan). Both sera were inactivated and absorbed twice mouse RBC and once with sheep RBC before use. Aliquots of cells (10% w/v spleen cell suspension pooled from 3 mice) were incubated for 30 min with RaM or NRS, respectively, at a final dilution of serum 1:50. Incubation was followed by 4 washings of cells in Hank's BSS before RFC assay. [b] Treatment of cell donors (3 per group): A) Immunization with 10^8 SRBC (–4 days); B) Infections with FLV (–5 days) and immunization with SRBC (–4 days); C) Infection with FLV (–5 days). [c] Cells pre-incubated with rabbit anti-mouse immunoglobulin serum. [d] Cells pre-incubated with normal rabbit serum. [e] No RFC found in total 10,000 spleen cells examined.

[6] N. K. JERNE, A. A. NORDIN and C. HENRY, in *Cell-Bound Antibodies* (Eds. B. AMOS and H. KOPROWSKI; Wistar Institute Press, Philadelphia 1963), p. 109.
[7] S. HIRANO and H. FRIEDMAN, Nature, Lond. *224*, 1316 (1969).
[8] T. BRODY, J. Immun. *105*, 126 (1970).
[9] H. WIGZELL, Transplant. Rev. *5*, 105 (1970).
[10] O. B. ZAALBERG, V. A. VAN DER MEUL and M. J. VAN TWISH, J. Immun. *100*, 451 (1968).
[11] J. CERNY, R. F. MCALACK and M. RICH, in preparation.
[12] G. L. ADA, Transpl. Rev. *5*, 105 (1970).
[13] A. H. COONS, in *Tolérance acquise et la tolérance naturelle à l'égard de substances antigeniques définies*, Colloques Internationaux de Centre National de la Recherche Scientifique (Paris 1963), p. 121.
[14] R. A. STEEVES and A. A. AXELROD, Int. J. Cancer *2*, 235 (1967).
[15] F. WILCOXON, in *Some Rapid Approximate Statistical Procedures* (American Cynanamid Company, New York 1949), p. 3.
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