Scanning electron microscopy of antibody-dependent cell-mediated cytotoxicity of Friend leukemia virus-infected spleen cells

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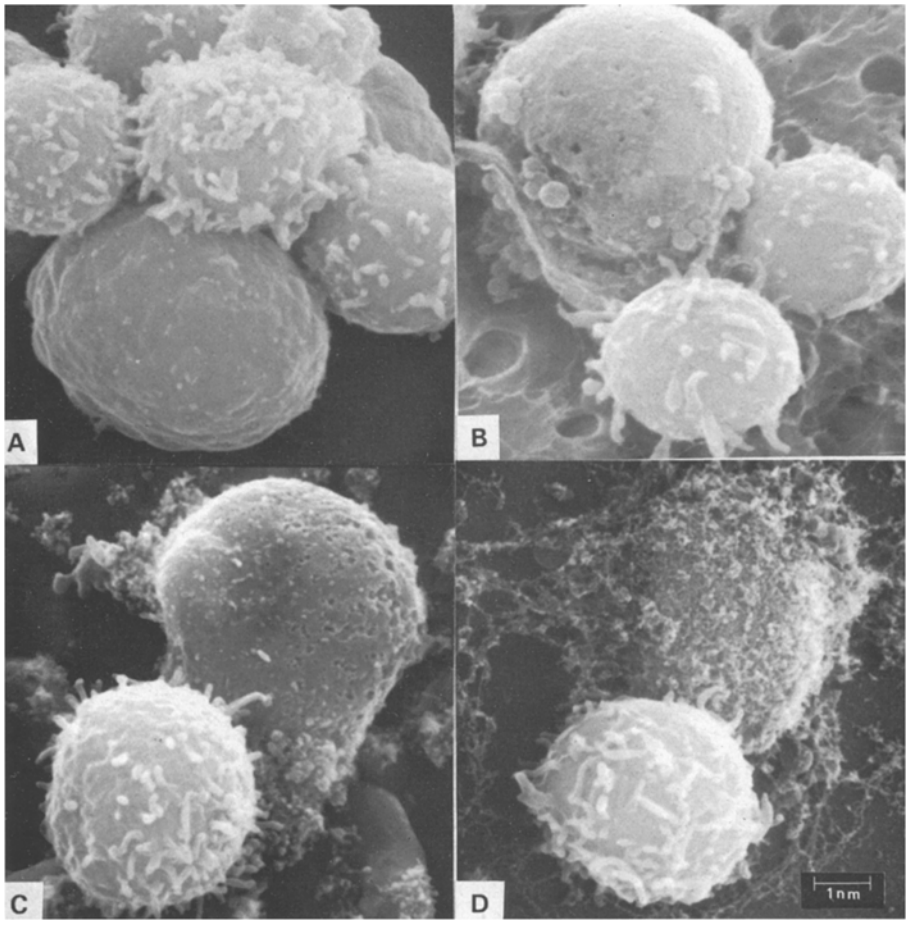
Summary. Friend leukemia virus (FLV) infected splenocytes treated with rabbit anti-FLV serum and subsequently incubated with splenic lymphocytes from non-immune Balb/c mice were examined by scanning electron microscopy. Villous-covered lymphocytes adherred to the tumor cells and induced surface blebs, numerous membrane pores and eventual tumor cell lysis.

Splenic lymphocytes from Friend leukemia virus (FLV)-infected mice have been examined by scanning electron microscopy[2,3]. Large, smooth-surfaced cells with small bumps appeared early in infection and progressively increased in numbers so that by 20 days after virus inoculation they comprised over 90% of the spleen cell population. Later in the course of infection many of these large cells were observed with blebs and pores in their membranes. These pores were thought to represent cell damage caused either by virus release or by immune-mediated phenomena such as cytotoxic antibodies or lymphocytes. We were interested in determining whether the surface modifications seen in the late stages of FLV-infection could be mimiced by an immune process. Antibody dependent cell-mediated cytotoxicity (ADCC) was utilized as a model system to study these alterations.

Material and methods. A stock preparation of FLV originally obtained from the American Type Culture Collection, Rockville, Maryland was used for infecting Balb/c male mice (Cumberland View Farms, Clinton, Tenn.). The virus was passaged in mice and consisted of a 10% clarified spleen cell homogenate. The preparation contained both the spleen focus-forming (SFFV) virus and the lymphatic leukemia (LV) virus components of the Friend complex. Anti-FLV serum was prepared by immunizing rabbits with FLV emulsified in complete Freund's adjuvant. Antisera thus obtained was heat-inactivated and absorbed twice with normal Balb/c spleen cells. The antiserum was capable of neutralizing 500 ID_{50} of virus at a dilution of 1:50. Leukemic cells were obtained from spleens of mice infected 21 days previously with FLV. At this stage of infection over 90% of splenocytes were of the large, smooth-surfaced variety previously described[2,3]. Leukemic and normal spleens were minced into small pieces, washed in cold tissue culture media, and purified by centrifugation in Ficoll-hypaque (Lymphoprep, Nyegaard and Co., Oslo, Norway). 2×10^6 leukemic cells were incubated with a 1:10 dilution of anti-FLV or normal rabbit serum in a volume of 1.0 ml at 4°C for 30 min. The serum-treated cells were washed in media and incubated with splenic lymphocytes



Scanning electron micrographs of leukemic cells undergoing antibody-dependent cell-mediated cytolysis.
A Villous-covered lymphocytes attaching to larger tumor cell; *B* 2 lymphocytes attaching to a tumor cell which has surface blebs and early signs of cytolysis; *C* lymphocyte attached to tumor cell which has numerous membrane pores; *D* lymphocyte attached to tumor cell which has lysed.
Scale bar: 1 nm.

from uninfected mice in a volume of 2.0 ml in 60-mm plastic petri dishes at a ratio of normal to leukemic cells of 100:1. The plates were placed in a tissue culture rotator and incubated for 60 min at 37°C. At the conclusion of the experiment the cells were centrifuged at low speed and washed twice with media and fixed with 1% glutaraldehyde. After 60-min fixation, the cells were allowed to settle onto Flotronic silver membranes (Selas Corp., Springhouse, Pa.) or Nucleopore filters (Nucleopore Corp., Pleasanton, Ca.). The cells were processed for scanning electron microscopy (SEM) as previously described[2,3] and examined with an Etec microscope (Etec Corp., Hayward, Ca.) operating at 20 kV.

Results and discussion. Leukemic cells were readily distinguishable from normal splenocytes by their larger size and smoother surface (figure, A). FLV-infected cells treated with anti-FLV or normal rabbit serum alone showed no evidence of morphologic changes. Incubation of normal spleen cells with tumor cells treated with normal rabbit serum also produced few changes in the appearance of the leukemic cells. Occasionally a tumor cell was seen with 1 or 2 lymphocytes adhering to its surface but no evidence of cytolysis was seen. When tumor cells which were preincubated with antiserum were mixed with normal spleen cells, a significant proportion of large smooth cells were observed with villous-covered lymphocytes adhering to their surface (figure, A). Tumor cells were seen in various stages of degradation (figure, B, C, D). Surface blebbing appeared on cells (figure, B) followed by the appearance of punctate lesions (figure, C) and eventual lysis (figure, D). More than 30% of tumor cells had villous covered lymphocytes attached to them, of these 12% showed signs of membrane damage, either by the production of blebs or pores. In addition, tumor cells with no attached lymphocytes were seen undergoing lysis.

The ability of lymphoid cells from normal individuals, who have not been sensitized to target cell antigens, to lyse those cells which have been incubated with antibodies against surface antigens is called antibody-dependent cell-mediated cytotoxicity (ADCC)[4]. In this report, the ability of lymphocytes from normal Balb/c mice to lyse FLV-transformed cells pre-treated with anti-FLV serum is demonstrated by SEM. The attachment of villous-covered lymphocytes results in tumor cell lysis. Similar changes were described in murine mastocytoma cells undergoing T-cell-mediated lysis[5]. These results indicate that the 'spongy' cells seen during the later stages of FLV infection might be the result of immunologic attack. However, this does not exclude the possibility that cytotoxic antibodies or virus release might produce the same morphologic changes. The effector cells for ADCC in other murine tumors has been designated as a K cell because of the absence of markers characteristic of either B or T sells 6. However, recent work by Lamon et al.[7] has suggested that the majority of splenic effector cells in ADCC are T cells. Such cells have receptors for either IgM or IgG and could bind with antibody-treated tumor cells. In humans, T-cells bearing receptors for IgM or IgG have been differentiated by electron microscopic appearance and surface morphology. The T-cell receptors of IgG (T_g) is more highly villous than the IgM receptor-bearing cells (Tm)[8]. Such differences have not been reported for murine T cells.

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2 P.A. Farber, S. Specter and H. Friedman, Science *190*, 469 (1975).
3 P.A. Farber, S. Specter and H. Friedman, Infect. Immun. *20*, 816 (1978).
4 A. Liepins, R.B. Faanes, J. Lifter, Y.S. Choi and E. de Harven, Cell. Immun. *28*, 109 (1977).
5 E.W. Lamon, H. Wigzell, E. Klein, B. Anderson and H.M. Skurzak, J. exp. Med. *137*, 1472 (1973).
6 A.H. Greenberg, L. Hudson, L. Shen and I.M. Roitt, Nature, New Biol. *242*, 111 (1973).
7 E.W. Lamon, B.R. Williams, E.W. Fuson, H.D. Whitten and A.S. Walia, J. Immun. *120*, 244 (1978).
8 C.E. Grossi, S.R. Webb, A.Zicca, P.M. Lydyard, L. Moretta, M.C. Mingari and M.D. Cooper, J. exp. Med. *147*, 1405 (1978).

Preparation of lymphocytes from small volumes of peripheral mouse blood[1]

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Summary. A method was developed which allowed for the isolation of lymphocytes (95% of leukocytes in the final suspensions) from small volumes of peripheral mouse blood (38% recovery). The method proved of value for studies of murine lymphocytes which required biochemical analysis of samples from individual animals on a serial basis.

The method described herein has proven of value in this laboratory for the study of lymphocytes in the limited volume of peripheral blood available from individual mice on a serial basis. It was developed by modification of previously reported techniques which require larger samples of blood[2-4]. Note that the term 'recovery', as used in this paper, denotes the relative proportion of cells which were present originally.

Materials and methods. Animals. Female SJL/J mice, 13 to 28 weeks old, were obtained from the closed breeding colony maintained in the Animal Services Center, University of Alabama in Birmingham.

Cell counts. Total leukocyte counts were determined in a Spencer hemacytometer chamber. The diluting fluid employed was 0.5% glacial acetic acid containing crystal violet. Differential leukocyte counts were made by examination of Wright-stained preparations. A minimum of 200 cells was counted in each smear.

Glassware. All glassware was siliconized by coating with Sigmacote (Sigma Chemical Corporation, St. Louis, Mo.).

Separation of lymphocytes. Blood samples (0.4 ml/mouse) were collected by tail-bleeding into heparinized tubes. Each sample was diluted with an equal volume of cold Hanks' balanced salt solution (H-BSS) and layered carefully with a