*Brief Definitive Report*

# SCANNING ELECTRON MICROSCOPY OF TOBACCO MOSAIC VIRUS-LABELED LYMPHOCYTE SURFACE ANTIGENS*

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The study of surface antigens by immunoelectron microscopy has been hampered by the fact that thin sections of cells provide only a view of the cell perimeter in an essentially two-dimensional fashion. Although the reconstruction of the entire cell from serial sections has been accomplished (1), it remains too exacting a technique and will find only exceptional application. Carbon-platinum replicas (2) allow the inspection of larger surface areas and therefore are better suited for studying the distribution of antigens (3). But since only relatively smooth surfaces will yield stable replicas, cells with large numbers of microvilli are not amenable to this technique.

Despite its limited resolution, scanning electron microscopy (SEM) seems to be the method of choice because it can provide a view of almost half of the surface of a cell close to its natural configuration, particularly after critical point or freeze drying (4, 5). Immunological-labeling methods have not yet been routinely applied to SEM although both latex spheres (6) and hemocyanin (7) have been used with some success. The optimal visual marker should possess the following properties: be of a distinctive shape, chemically stable, and have per se a low binding affinity for cell surfaces. Tobacco mosaic virus (TMV), a marker with which we are familiar in transmission electron microscopy (8), seems to meet these demands; it has rod-like shape and defined dimensions (15 × 300 nm) and in addition it can easily be distinguished from surface microvilli. As the hybrid antibody technique (9) is also applicable to TMV, we have attempted to combine such immunological labeling with SEM. We present evidence that surface antigens can indeed be visualized by SEM, using the TMV marker in conjunction with the hybrid antibody technique.

## Material and Methods

A general description of the hybrid antibody technique has been previously reported (10). Murine lymphocytes (lymph node or thymus cells) were labeled in suspension by the sequential incubation at 4°C in mouse alloantibody (e. g., a 1:20 dilution anti-Thy-1, anti-Ia (11), or 1:40 diluted anti-H-2 antibody, respectively), wash medium, hybrid anti(a)-TMV/aIgG antibody (3) at 50–100 μg/ml, wash medium, and finally TMV. After the final wash the cells were collected by aspiration onto silver membranes (12) and processed for SEM using the critical point drying technique (13-15). Samples were inspected in a Cambridge S4 (Cambridge Thermionic Corp., Cambridge, Mass.) or Jeol JSM-35 scanning electron microscope (courtesy of Jeol U. S. A., Electron Optics Div., Medford, Mass.)

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## Discussion and Results

Scanning micrographs revealed the presence of TMV as long rods, some of which appeared perpendicular to the cell surface, while others were bent or curled (Figs. 1 and 5). The length of the rods varied in our TMV preparation due probably to breakage during thawing of deep frozen stored material. Furthermore the apparent length of TMV rods varies according to the angle of view, and could only be accurately measured on stereopair pictures. Consequently length cannot be used as a reliable criterion to distinguish TMV from cellular microvilli. Fortunately on the basis of their constant width which is mostly independent of their orientation, TMV were easily identified in most cases, even on villous lymphocytes (Fig. 1) despite the presence of relatively large numbers of microvilli (15–19).

The serological specificity of labeling was established in control preparations which yielded only very little labeling or none at all. These controls included: (*a*) substitution of anti-Thy-1.1 for anti-Thy-1.2 antiserum to label C57BL/6 thymocytes; (*b*) substitution of ASW strain lymphocytes for A-strain lymphocytes in Ia-antigen labeling; (*c*) omission of hybrid antibody; and (*d*) omission of marker. TMV antigens were not seen in most controls, with the exception of the ASW control for Ia where a consistent but sparse labeling was observed. We surmise that this background labeling is due to cross-reaction of the hybrid aIgG/aTMV with B-cell surface immunoglobulin, but as this aspect is dealt with in detail in a separate report, we shall not elaborate on it here.[1]

The study of Thy-1 and Ia antigens seemed to be an excellent choice because the two antigen systems could be expected to be represented on different cells, namely Thy-1 on T lymphocytes and Ia predominantly on B lymphocytes (11). As seen in Fig. 2, Thy-1 treatment of lymph node cells results in almost selective, dense labeling of the smooth category of lymphocytes which are believed to represent T cells. Cells with multiple microvilli, most of which are believed to be B lymphocytes, did not carry significant numbers of TMV (Fig. 2), but occasionally densely labeled villous cells were found. Again, we can invoke surface Ig to explain attachment of TMV to villous cells. On the other hand, it is possible that they represent moderately villous T cells (15–19). Hybrid antibody with exclusive gamma-chain specificity such as aIgG-Fc/aTMV is needed to avoid cross-reactivity with surface Ig, and a recent experiment with this hybrid antibody indeed shows a higher degree of specificity, i. e., no detectable labeling of surface Ig was seen by transmission electron microscopy.

When lymph node lymphocytes were coated with anti-Ia antibody which is selective for B cells (11), we find the TMV on approximately one-third of the population (Fig. 3), in accordance with the known proportion of lymph node B cells. Most of the unlabeled ($Ia^-$, $Ig^-$) cells had a relatively smooth surface confirming our previous findings that T lymphocytes, under the conditions employed, display a "smoother" surface architecture. However, the labeled $Ia^+$ $Ig^+$ population consisted of villous cells (Fig. 1) in somewhat lower numbers than

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[1] Lipscomb, M. F., K. V. Holmes, E. S. Vitetta, J. W. Uhr, and U. Hämmerling. 1975. Localization of lymphocyte surface immunoglobulin by scanning electron microscopy. *Eur. J. Immunol.* In press.

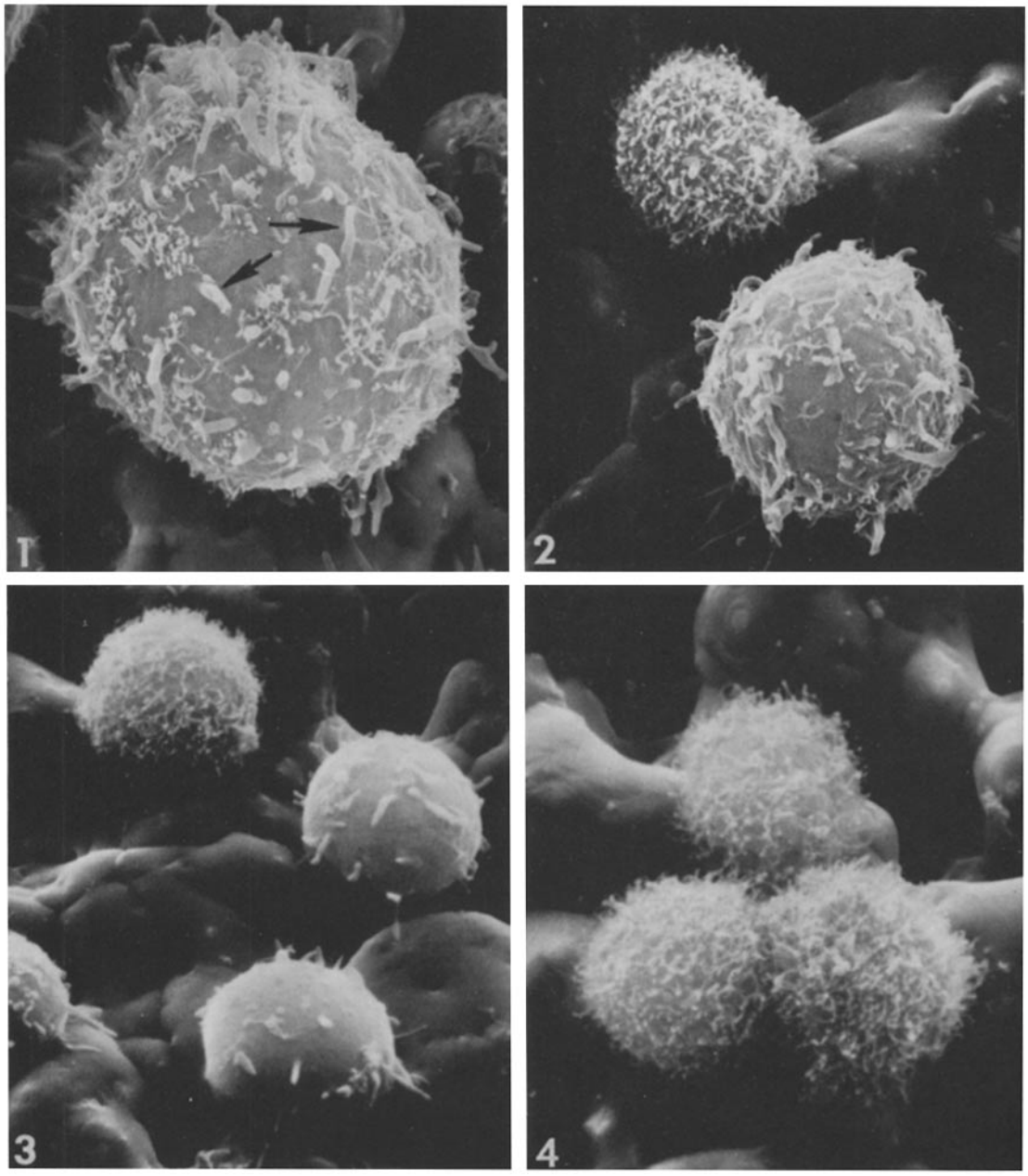


FIG. 1. Ia antigen on lymphocytes. (C57BL/6 x A) $F_1$ lymph node cells were treated with: (ATH x B10S) $F_1$ anti-(ATL x B10S) lymphocytes (courtesy of G. Hämmerling and H. O. McDevitt) ("congenic anti-Ia"); hybrid antimouse IgG/anti-TMV; and TMV. Villous B cell showing dense labeling with TMV. The difference between microvilli (arrows) and the narrower rod shaped TMV is easily discernible (× 18,000).

FIG. 2. Thy-1 antigen on lymphocytes. Lymph node cells of C57BL/6 mice were labeled in succession with the serum: (A/Thy-1.1 x AKR/*H-2b*) $F_1$ anti-A-strain leukemia ASL 1 ("congenic anti-$\theta$"); hybrid antimouse IgG/anti-TMV; and TMV. The smaller smooth T lymphocyte is diffusely labeled with TMV. Some of the TMV are bent and curled, others are

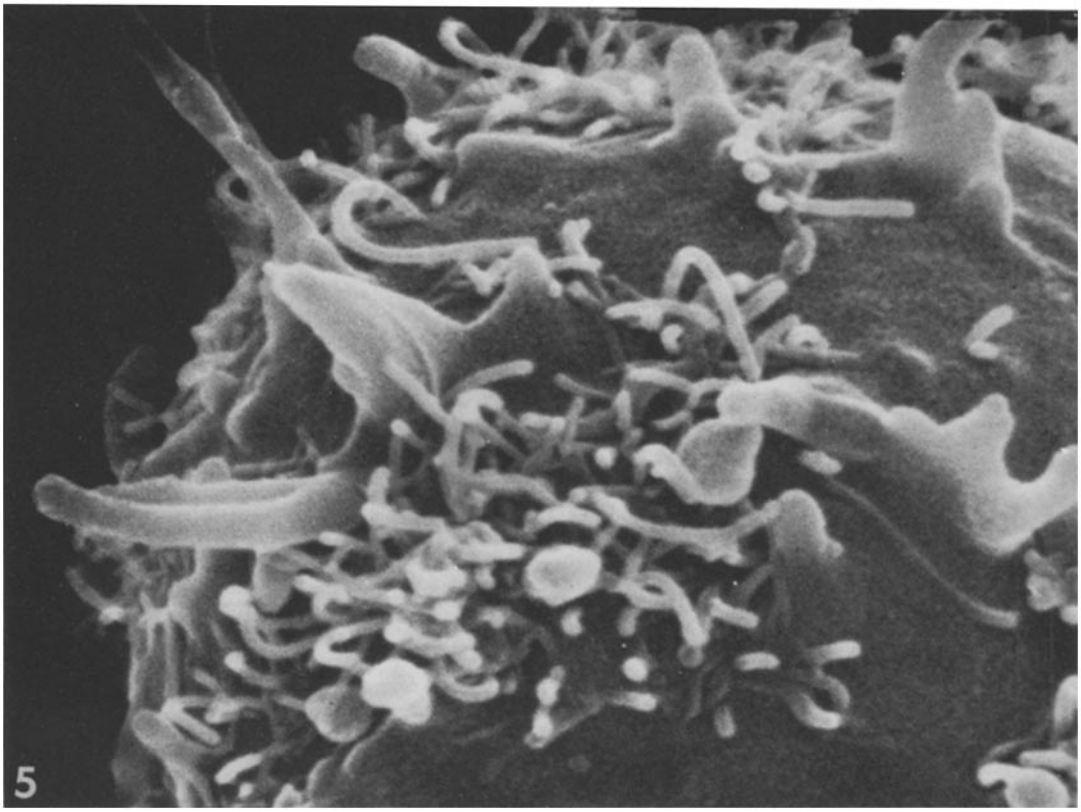


FIG. 5. Thy-1 antigen on lymphocytes. Lymph node cells (treated as in Fig. 1), showing narrow rod-shaped TMV which are easily distinguished from the broader microvilli. Note the appreciably better resolution of this micrograph, taken recently with a more advanced type of SEM (courtesy of Jeol U. S. A., Electron Optics Div., Medford, Mass.) (× 42,000).

expected, and a substantial number of labeled cells with smoother morphology was also seen (Fig. 3). Three possible explanations can be advanced. Firstly, a proportion of T cells may express the Ia antigen. Secondly, not all B cells necessarily have a villous appearance, as was indeed suggested in previous reports (17, 19) where a proportion of murine B cells purified in two independent ways were smooth or displayed only moderate numbers of microvilli. Thirdly, the process of attachment of layers of antibodies and marker may cause some cells to re-

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more erect, and a few have collapsed onto the cell surface. The larger villous cell has scanty labeling, and is presumably a B lymphocyte. (The small number of TMV apparent between the microvilli on this cell may identify surface Ig.) (× 8,000).

FIG. 3. Ia antigen on lymphocytes. Lymph node cells treated as described in Fig. 1 Only one of the three cells is labeled with TMV. Labeling is of the diffuse type and microvilli are not evident. In these preparations both "villous" and "smooth" cells labeled with the B-cell marker (× 6,600).

FIG. 4. *H-2* antigen on lymphocytes. C57BL/6 lymph node cells were labeled with the serum: [C57BL/6 (*H-2K*) x AKR]$F_1$ anti-C57 strain leukemia EL 4 ("congenic anti-*H-2b*"); hybrid antimouse IgG/anti-TMV, and TMV. Note the diffuse dense labeling of all cells with TMV (× 7,000).

tract their microvilli. The latter effect was particularly striking in lymph node cells labeled with anti-*H-2* antibody. In this case, the overwhelming majority of cells were labeled in a diffuse pattern covering the entire surface (see Fig. 4), and although it was often difficult to discern microvilli among the maze of TMV we felt that a deficit existed in the expected minimum proportion of villous cells, suggesting a loss of microvilli. That this can indeed occur followed from a series of experiments in which lymphocytes were exposed at 0° to anti-*H-2* serum or anti-Thy-1.2 serum alone. Cells so treated showed alterations of surface morphology, resulting at times in a decrease in the number of villous cells. It is possible that these effects are due to low temperature (20), or perhaps to forces exerted by strongly cross-linking antibodies.

The hybrid antibody technique, employing TMV as a marker, can thus be applied to labeling SEM with adequate immunolocial specificity. This can be of significance in the investigation of the distribution of cell surface antigens, and may in turn contribute to resolving the question of whether alloantigens are largely mobile within the lipid matrix of the plasmalemma (21), or whether restrictions are imposed on mobility, and areas of predominant representation of alloantigens can be discerned.

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