Proc. Natl. Acad. Sci. USA
Vol. 78, No. 5, pp. 3170–3174, May 1981
Immunology

Expression and synthesis of murine immune response-associated (Ia) antigens by brain cells

(major histocompatibility complex/immunoperoxidase/immunoprecipitation)

JENNY P. Y. TING, BRIAN L. SHIGEKAWA, D. SCOTT LINTHICUM, LESLIE P. WEINER, AND JEFFREY A. FRELINGER

Departments of Microbiology and Neurology, University of Southern California Medical School, 2025 Zonal Avenue, Los Angeles, California 90033

Communicated by Ray D. Owen, January 27, 1981

ABSTRACT **This paper provides biochemical and histochemical evidence that a fraction of murine brain cells express and synthesize Ia (*I*mmune response-*a*ssociated) antigens. Both I-A and I-E subregion products are detected on frozen sections of mouse brains by immunoperoxidase staining. Most of these Ia-bearing cells are located in white matter tracts and appear to be intrafascicular oligodendrocytes. In contrast, cells in the gray matter rarely display detectable Ia antigens on their cell surfaces. Specificity of the staining was confirmed by absorption studies. Biochemical evidence for the active synthesis of Ia antigens by brain cells was obtained by immunoprecipitation of [^{3}H]leucine/tyrosine-labeled, NP-40-extracted cell lysates with monoclonal anti-Ia reagent. Both the α and β subunits of Ia antigens were identified by $NaDodSO_4$ electrophoresis. By contrast, anti-μ serum failed to precipitate any product, thus eliminating contaminant B lymphocytes as a source of Ia antigens.**

The role of immunological mechanisms in the central nervous system has stimulated much interest among neurologists and immunologists. The blood brain barrier and the lack of lymphatic vessels appears to be fairly efficient in excluding circulating cells and proteins in blood from entering this system (1). This leads to the assumption that the central nervous system represents an immunologically privileged site; however, many disorders of this system are correlated with abnormal immune responses that may be fundamentally autoimmune in nature. Studies of acute disseminated encephalomyelitis suggest that cellular immunity to myelin basic protein can result in central nervous system lesions (2). Likewise, immunological abnormalities are frequently detected in multiple sclerosis patients. These include inflammatory demyelination of the central nervous system, the presence of oligoclonal immunoglobulins in the cerebrospinal fluid, and less than normal suppressor T-cell activity (3–6). Therefore, the pertinent question is whether the central nervous system has endogenous immunologic capacities that are responsible for such autoimmune-like phenomena. We approach this question by examining murine brains for cells that bear Ia antigens.

The major histocompatibility complex of the mouse (*H-2*) is divided into four major regions, *K*, *I*, *S*, and *D* (7). The *I* region regulates immune responses and is divided into five subregions by a combination of serological and functional analyses. Genes mapping within the *I* region code for dimeric membrane glycoproteins called Ia antigens (8). Although a direct relationship between genes regulating immune responsiveness and those coding for the Ia antigens is not firmly established, current evidence indicates a central role for Ia-bearing cells in immune functions. Unlike the widely distributed classical transplantation antigens mapping in the *K* and *D* regions, surface Ia antigens have limited tissue distribution and are found predominantly on cells involved in immune responses. B lymphocytes and subpopulations of T lymphocytes are known to have surface Ia antigens (9–11). Accessory cells that participate in antigen presentation also express Ia determinants (12–15). In addition, Ia-positive cells in liver and skin have a similar antigen presenting function (16, 17). Hence, Ia-positive cells from both lymphoid and nonlymphoid tissues are associated with immune function, and the presence of Ia antigens might be a marker for immune competency. Therefore, we reasoned that the identification of Ia-bearing cells in a specific tissue or organ may lead to the elucidation of immune mechanisms within that tissue or organ. In this study, we investigated brain tissues because of their importance as targets of immune-mediated diseases.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIAL AND METHODS

Mice. All mice used were bred in our animal facilities at the University of Southern California.

Antisera. Conventional alloantisera were produced by repeated immunization of appropriate recipient mice with a combination of spleen, thymus, and lymph node cells as described (18). The antisera used are shown in Table 1. The monoclonal hybridoma, 10–2.16 developed by Oi *et al.*, was obtained from the Salk Institute, La Jolla, CA (19). The anti-μ serum was a gift from Richard Douglas, California Institute of Technology, Pasadena, CA. Alloantisera were characterized by cytotoxicity testing on inbred and recombinant animals before use.

Immunoperoxidase Staining. Mouse brains were fixed overnight with 10 mM $NaIO_4$/75 mM lysine/2% paraformaldehyde (20). Frozen sections (8-μm thick) were prepared with a cryostat. These sections were pretreated with 0.6% H_2O_2 diluted in absolute methanol for 30 min to inhibit endogenous peroxidase. They were then washed with phosphate-buffered saline and treated with 1:20 dilution of normal horse serum. This step decreases nonspecific binding of the secondary reagent. The sections were sequentially incubated with primary antiserum, horse anti-mouse IgG antibodies conjugated with biotin, and avidin-peroxidase (the latter two reagents were purchased from Vector Labs, Burlingame, CA). Slides were washed extensively between each incubation. A solution of diaminobenzidine (0.6 mg/ml) and H_2O_2 (0.01%) was then added to the sections. Immunoperoxidase reactivity was visualized by the oxidation of diaminobenzidine to form a brown product.

Biosynthetic Labeling and Immunoprecipitation. Spleen cells were prepared and labeled as described (21). Dissociated brain cells were prepared by a modification of published methods (22). Briefly, each animal was perfused at a constant pressure of 3 psi (1 psi = 6.89 kPa) with 150 ml of phosphate-buffered saline/2% (wt/vol) glucose/0.02% collagenase/0.10% hyaluronidase/1% (wt/vol) bovine serum albumin. After per-

Table 1. Presence of Ia-positive brain cells in different mouse strains

Exp	Strain	Major histocompatibility complex: K	I region: A	I region: B	I region: J	I region: E	I region: C	S	D	Primary antiserum	Antiserum specificity	Immunoperoxidase reactivity
1–2	B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	A.TL anti-A.TH	I^s	+
										A.TH anti-A.TL	I^k	–
	B10.K	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	A.TL anti-A.TH	I^s	–
										A.TH anti-A.TL	I^k	+
	B10.HTT	*s*	*s*	*s*	*s*	*k*	*k*	*k*	*d*	A.TH anti-A.TL	I^k	+
										A.TL anti-A.TH	I^s	+
										Normal mouse serum	None	–
3–6	AQR	*q*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	(A.TH × B10.D2)F_1 anti-A.TL	I^k	+
										A.TL anti-A.TH	I^s	–
	B10.T(6R)	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*d*	(A.TH × B10.D2)F_1 anti-A.TL	I^k	–

fusion, brains were rapidly removed, minced into 1-mm^3 pieces, and incubated in the perfusion medium at room temperature for an additional 5 min. Cells were dissociated by gentle pipetting using a Pasteur pipette, and the subsequent suspension was passed through a Nitrex 355 nylon filter (Tetko, Monterey Park, CA) to remove tissue debris and cell aggregates. The preparation was washed three times and suspended in Hanks' balanced salt solution. Preparations from each animal were examined microscopically for blood contamination and only those with <5% erythrocytes were pooled and used. Cell viability was 65–75%. For radiolabeling, 2×10^7 spleen or brain cells were cultured in balances salt solution/10 mM Hepes/5% dialyzed fetal bovine serum containing [^{3}H]leucine and [^{3}H]tyrosine at 1 mCi/ml (1 Ci = 3.7×10^{10} becquerels). Preparations were labeled for 6 hr at 37°C in 5% CO_2/95% air, washed, and extracted with NP-40. Extracts were passed through a 10-ml *Lens culinaris* lectin column, immunoprecipitated with the appropriate antibody and protein A-bearing *Staphylococcus aureus* (Cowan I strain) and subjected to electrophoresis on one-dimensional $NaDodSO_4$/polyacrylamide gels as described (21).

RESULTS

We used a triple-layered immunoperoxidase technique to reveal Ia-positive cells in the brain (Fig. 1). The basic criterion used for scoring positive cells is the presence of a brown enzymatic product on the cell membrane. The stained slides were routinely read blind by at least two individuals and all readings were concordant. The Ia^+ population identified by this method constitutes a minor fraction (<1%) of the central nervous system cell population. Although one cannot be certain at the light microscopic level of the precise identification of these cells, the morphology and anatomical localization of these Ia^+ cells suggest that they may be oligodendrocytes. Approximately 90% of the Ia-bearing cells are found in the white matter tracts and are lined up in a fashion that is reminiscent of intrafascicular oligodendrocytes. The remainder of the Ia^+ cells are scattered through the grey matter or are localized in the white matter but cannot be identified. These specifically stained cells could be oligodendrocytes, astrocytes, microglia, or the undifferentiated third type glia recently identified in rodents (23). In contrast, in all the slides we have examined, the neuronal layers that are easily identified by light microscopy do not stain with anti-Ia reagents. This is true of the cerebellum, cerebrum, and brain stem. We performed the following experiments to establish the presence of Ia^+ brain cells in various mouse strains (Table 1). Stained cells were identified in B10.S (I^s) brain sections incubated with anti-I^s serum (A.TL anti-A.TH) but not with an irrelevant anti-Ia serum (A.TH anti-A.TL). When B10.K brain sections were similarly prepared and examined, specific staining could be demonstrated with anti-I^k serum but not with anti-I^s serum. Similarly, Ia-bearing cells were identified in AQR (I^k) brains, when treated with a specific antiserum, but not with an irrelevant serum.

Because all the Ia-bearing cell populations thus far identified in other organs and tissues express both I-A and I-E subregion products, it was of interest to determine whether Ia-bearing brain cells also express these subregion products (8, 14, 15, 24). We used the recombinant strain B10.HTT, which is $I\text{-}A^s$ and $I\text{-}E^k$, for these experiments (Table 2). Antisera directed at I^s products will react with the I-A subregion product of B10.HTT, and those directed at I^k products will react with the I-E subregion products of B10.HTT cells. Both of these antisera resulted in specific straining. Normal mouse serum showed no specific staining (Table 1). Hence, both I-A and I-E are expressed by these cells. This paralleled the expression of Ia surface products on other Ia-bearing cells.

Although the sera used in these experiments have been extensively analyzed by direct cytotoxicity and absorption, it was critical to test our immunohistochemical procedure and show that the specific reactivity was directed only at *I* region products. The following experiment was designed to study this problem. We absorbed the A.TL anti-A.TH serum with A.SW (I^s) spleen cells to remove antibody reactivity directed at I^s products. Another aliquot of the antiserum was preabsorbed in an identical fashion with B10.K (I^k) splenocytes to serve as a negative absorption control. The resultant serum preparations were tested on B10.S brain sections. Absorption with A.SW cells specifically removed reactivity previously observed in brain sections, while preincubation with B10.K cells had no effect on the staining of B10.S sections (Table 2). Similar results were demonstrated with AQR brain sections. Preabsorption of an antiserum that has anti-I^k activity with cells expressing I^k products specifically removed staining reactivity on AQR (I^k) sections, while preincubation with cells expressing I^q products had no effect. Hence, these results indicate that the antisera were indeed detecting I region products and not some unrelated cell surface entities.

Although reactivity of the antisera against non-Ia cell surface components (e.g., Tla, Qa-1, and such) could have been a problem, several lines of evidence rule this out. B10.S and B10.K share the same Tla^b type, but reacted antithetically. Most convincingly, both B10.T(6R) and AQR share their *TL* region by derivation from strain A, but AQR stained with serum (A.TH×B10.D2)F1 anti-A.TL, while B10.T(6R) failed to do so. Similarly, B10.T(6R) failed to absorb the activity in this serum for AQR brain sections. Thus these observations rule out map-

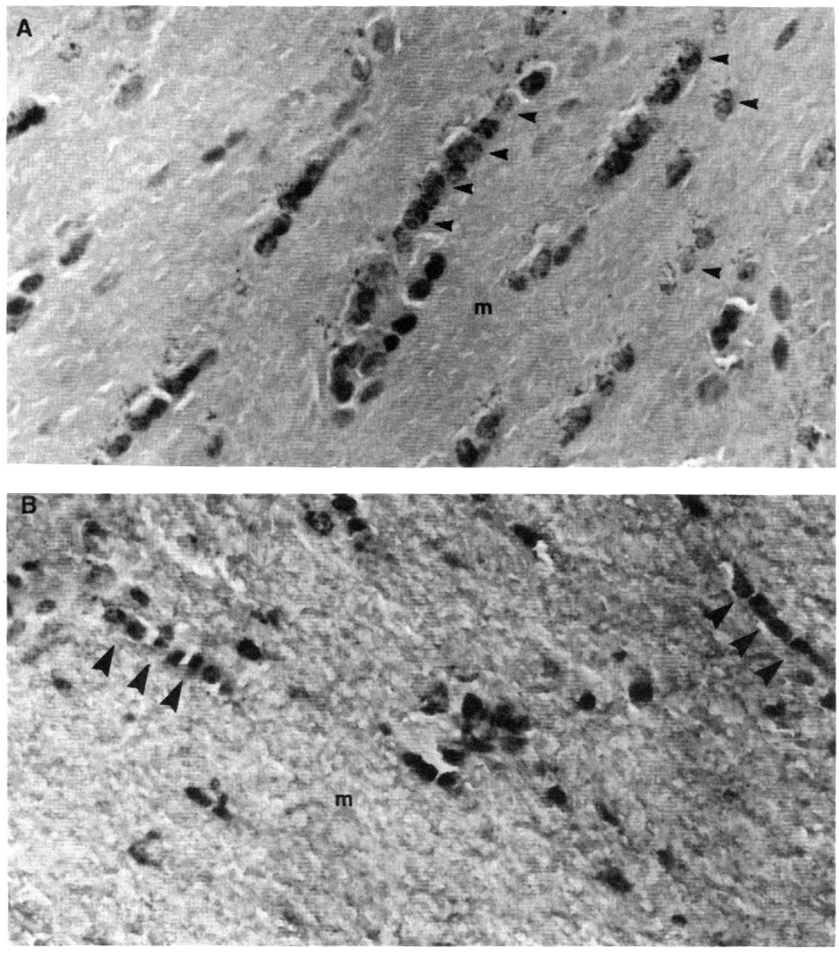

FIG. 1. Immunoperoxidase staining of Ia-bearing cells in the white matter tract of mouse brains. Frozen sections of murine brains were sequentially treated with A.TL anti-A.TH serum, horse anti-mouse IgG bound to biotin, and horseradish peroxidase-avidin. (*A*) White matter tract of the positive B10.S mouse. Small arrows indicate Ia^+ immunoperoxidase-labeled cells. The stained cells have brown products on their surface that are apparent on colored prints. (*B*) Similar area of the negative control, B10.K brain, treated with the same antiserum. There are no positive cells. Equivalent cells are indicated by large arrows. Note that the unlabeled cells appear smaller because they were stained by the nuclear stain hematoxylin and, in the absence of cell surface immunoperoxidase reaction products, only the nucleus is visible. m, myelin. (×800.)

ping of this reaction telomeric to *H-2D*.

Despite the serological controls that map the reactivity on brain sections to the *I* region, it was still possible that the sera were reacting with a previously unknown class of molecules in the brain. A preliminary chemical characterization of the brain Ia was carried out. Fig. 2 shows the $NaDodSO_4$/acrylamide gel patterns of Nonidet P40 extracts of B10.A brain cells immunoprecipitated with anti-D^d, I-A^k, or μ reagents. Spleen cells from the same mice were run in parallel as positive controls. When brain cell lysate was treated with anti-D^d serum, a peak of ≈45,000 daltons that comigrated with spleen H-2D was obtained. More importantly, monoclonal antibody directed at I-A^k precipitated two characteristic molecular species of ≈31,000 and 28,000 daltons that had migration patterns indistinguishable from Ia antigens isolated from splenocytes. This demonstrates that Ia antigens in the brain are similar if not identical to Ia antigens observed in spleen cells. These data further rule out the possibility that the immunoperoxidase reactivity described above is due to TL region molecules. Only peptide mapping and amino acid sequence analysis could provide more

Table 2. Determination of anti-Ia specificity by absorption with splenocytes

Strain	Major histocompatibility complex *I* region								Primary Antiserum	Antiserum Specificity	Cells used for Absorption	Immunoperoxidase reactivity
	K	*A*	*B*	*J*	*E*	*C*	*S*	*D*				
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	A.TL anti-A.TH	I^s	None	+
											A.SW (I^s)	−
											B10.K(I^k)	+
AQR	*q*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	(A.TH × B10.D2)F_1 anti-A.TL	I^k	None	+
											B10.K(I^k)	−
											B10.T(6R) (I^q)	+

Antiserum (0.2 ml of a 1:20 dilution with phosphate-buffered saline) was absorbed with 10^7 splenocytes of the designated strain for 30 min at 4°C.

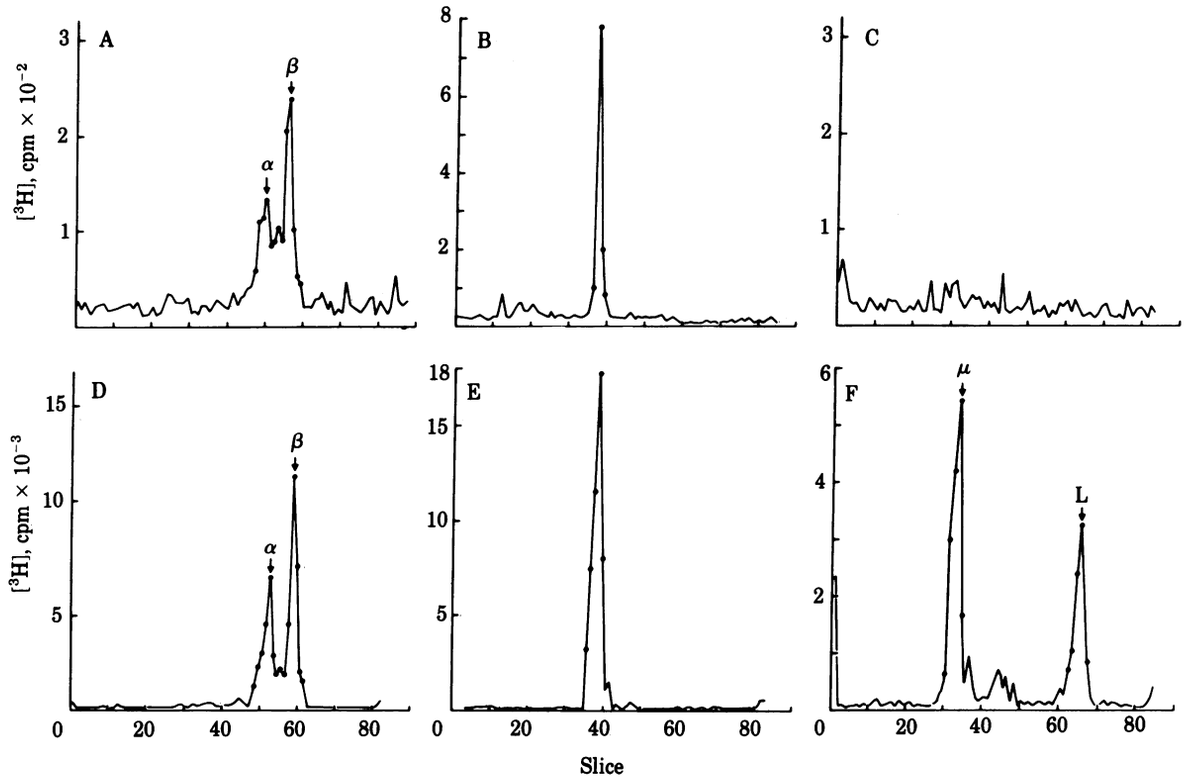

FIG. 2. $NaDodSO_4$/polyacrylamide gel electrophoresis patterns of Ia and H-2D molecules isolated from brains (*A–C*) and spleens (*D–F*). Lectin-purified B10.A brain and spleen lysates were immunoprecipitated with antibody and *Staphylococcus aureus*. (*A* and *D*) I-A^k molecules detected by monoclonal antibody 10.2-16. (*B* and *E*) D^d molecules detected by [B10.A(1R) × A.SW]F1 anti-B10.A serum. (*C* and *F*) Molecules detected by anti-μ serum. The last slice of each gel is the dye front.

definitive information on the nature of the brain Ia molecules.

Because it was crucial to establish that the Ia antigens are actually produced by cells in the brain and not contaminating blood cells, we took the precaution of perfusing extensively and selecting cell preparations having <5% erythrocyte contamination. Assuming an erythrocyte/leukocyte ratio of 500:1 in the blood, there should be less than 2×10^3 leukocytes in 2×10^7 brain cells, and this small number would not result in a demonstrable Ia peak. The biochemical data confirmed this assumption that B lymphocytes were not the source of Ia antigens in brain cultures; anti-μ serum did not precipitate any product (see Fig. 2 *E* and *F*). Further, as the μ peak in the spleen is nearly the same size as the α peak, a μ peak similar to the Ia peak should have been detected if the brain Ia were derived from B lymphocytes.

DISCUSSION

Our results indicate the presence of Ia-bearing cells in the brain. The functional role of these cells is unknown. However, taken in context with current concepts on the role of Ia-bearing accessory cells identified in different tissues, the implications are important. The function of different Ia^+ subpopulations has recently received much attention. Among lymphoid organs, Ia^+ accessory cell populations have been shown to be the most potent stimulators in *in vitro* allogeneic mixed-lymphocyte reactions and soluble-antigen presentation systems (12–15). This population may include both macrophages and splenic dendritic cells, although this still remains a debatable issue. Ia-bearing macrophages and dendritic-like cells have also been identified in the thymus (25, 26). Among nonlymphoid organs and tissues, Ia-bearing Kuppfer cells in the liver and Langerhans cells in the skin have been well studied (27–29). Both of these cell types are capable of *in vitro* antigen presentation. In addition, both Kuppfer cells and Langerhans cells appear to have a hematopoietic origin, which further supports their macrophage-like characteristics. Thus, an attractive hypothesis is that the different Ia-positive subpopulations are putative tissue macrophages and that the expression of Ia antigens on these cells is crucial for immunological functions.

Our current results are especially pertinent because immune mechanisms in the central nervous system are not well understood. Based on observations with other Ia-bearing accessory cells, one can extend those observations and speculate that the Ia-positive brain cells may also function in antigen presentation, and perhaps other accessory cells since demyelination and the destruction of oligodendrocytes are believed to be the primary pathologic mechanism in multiple sclerosis (30). Therefore, further study of the relationship of these cells and their role in demyelination and antigen processing will be of considerable clinical significance.

We thank Dr. Rich Harmon for critical reviews, Ray Russell for helpful suggestions, and Nancy Harper and Jennifer Lee for preparing the antisera. This work was supported by National Institutes of Health Grants CA 22662 and NS 12967. J.P.Y.T. is supported by National Institutes of Health National Research Service Award Grant NS 07149, B.L.S. is an American Cancer Society Postdoctoral Fellow, D.S.L. is a Leukemia Society of America Scholar, and J.A.F. is the recipient of American Cancer Society Faculty Research Award 179.

1. Westergaard, E. (1977) *Acta Neuropathol.* **39**, 181–187.
2. Lisck, R. P. (1974) *Neurology* **24**, 560–564.
3. Johnson, R. T. (1975) *Adv. Neurol.* **13**, 1–46.
4. Haire, M. (1977) *Br. Med. Bull.* **33**, 40–44.
5. Arnason, B. G. W. & Antel, J. (1978) *Ann. Neurol.* **129**, 11–14.

6. Link, H. & Tibbling, G. (1977) *Scand. J. Clin. Lab. Invest.* **37,** 397–401.
7. Klein, J. (1975) *Biology of the Mouse Histocompatibility-2 Complex* (Springer, New York), pp. 389–489.
8. Shreffler, D. C. & David, C. S. (1975) *Adv. Immunol.* **20,** 125–195.
9. Hammerling, G. J., Deck, B. D., Mauve, G., Hammerling, U. & McDevitt, H. O. (1974) *Immunogenetics* **1,** 68–81.
10. Frelinger, J. A., Niederhuber, J. E., David, C. S., Shreffler, D. C. (1976) *J. Exp. Med.* **140,** 1273–1284.
11. Murphy, D. B., Herzenberg, L. A., Kumura, K. O., Herzenberg, L. A. & McDevitt, H. O. (1976) *J. Exp. Med.* **144,** 699–712.
12. Cowing, C., Pincus, S. H., Sachs, D. H. & Dickler, H. B. (1978) *J. Immunol.* **121,** 1680–1686.
13. Steinman, R. M. & Witmer, M. D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5132–5136.
14. Cowing, C., Schwartz, B. D. & Dickler, H. B. (1978) *J. Immunol.* **120,** 378–384.
15. Sunshine, G. H., Katz, D. R. & Feldman, M. (1980) *J. Exp. Med.* **152,** 1817–1822.
16. Stingl, G., Katz, S. I., Shevach, E. M., Wolff-Schreiner, G. & Green, I. (1978) *J. Immunol.* **120,** 570–578.
17. Richman, L. K., Klingerstein, R. J., Richman, J. A., Strober, W. & Berzofsky, J. A. (1979) *J. Immunol.* **123,** 2602–2609.
18. David, C. S., Shreffler, D. C. & Frelinger, J. A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2509–2514.
19. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberrg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81,** 115–129.
20. McLean, I. W. & Nakane, P. K. (1974) *J. Histochem. Cytochem.* **22,** 1077–1084.
21. Cullen, S. E., David, C. S., Shreffler, D. C. & Nathenson, S. G. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 648.
22. Althaus, H. H., Huttner, W. B. & Neuhoff, V. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358,** 1155–1159.
23. Vaugh, J. E. & Peters, A. (1968) *J. Comp. Neurol.* **133,** 269–274.
24. Frelinger, J. G., Wettstein, P. J., Frelinger, J. A. & Hood, L. (1978) *Immunogenetics* **6,** 125–135.
25. Rouse, R. V., Van Ewijk, W., Jones, P. P. & Weissman, I. L. (1979) *J. Immunol.* **122,** 2508–2515.
26. Beller, D. I. & Unanue, E. R. (1980) *J. Immunol.* **124,** 1433–1440.
27. Van Furth, R., Crofton, R. W. & Diessdhoft-den Dulk, M. M. C. (1977) in *Kupffer Cells and Other Liver Sinusoidal Cells,* eds. Wisse, E. & Knook, D. L. (Elsevier/North-Holland, New York), pp. 471–480.
28. Frelinger, J. G., Hood, L., Hill, S. & Frelinger, J. A. (1979) *Nature (London)* **282,** 321–323.
29. Katz, S. I., Tamaki, K. & Sachs, D. H. (1979) *Nature (London)* **282,** 324–326.
30. Lampert, P. W. (1978) *Am. J. Pathol.* **91,** 176–208.