Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 1506–1510, March 1982
Biophysics

H-2K^k and vesicular stomatitis virus G proteins are not extensively associated in reconstituted membranes recognized by T cells

(photobleaching/liposomes/H-2 restriction/fluorescence)

GENE S. CARTWRIGHT*†, LLOYD M. SMITH*, ERIC W. HEINZELMANN‡, MARY J. RUEBUSH‡, J. WALLACE PARCE§, AND HARDEN M. MCCONNELL*¶

*Stauffer Laboratory for Physical Chemistry, Stanford University, Stanford, California 94305; and ‡Department of Microbiology and Immunology, and §Department of Biochemistry, Bowman Gray School of Medicine, Winston–Salem, North Carolina 27103

Contributed by Harden M. McConnell, December 10, 1981

ABSTRACT **It is shown that liposomes containing (*i*) a fluorescein-labeled murine histocompatibility antigen (FITC-H-2K^k) and the G protein of vesicular stomatitis virus or (*ii*) H-2K^k and fluorescein-labeled viral protein (FITC-G) can elicit *H-2*-restricted syngeneic antiviral cytotoxic T cells as assayed by ^{51}Cr release from appropriate virus-infected target cells. Fluorescence recovery after photobleaching was used to measure the diffusion coefficients of these reconstituted proteins in four different samples: (*i*) FITC-H-2K^k; (*ii*) FITC-H-2K^k and G; (*iii*) FITC-G; and (*iv*) FITC-G and H-2K^k. The same rate of lateral diffusion ($D = 1 \times 10^{-8}$ cm^2/sec at 37°C in 25% cholesterol/75% dimyristoylphosphatidylcholine) was obtained in every case. Both proteins, fluorescent as well as nonfluorescent, could be patched by using specific antibodies. When G was patched with antibody, FITC-H-2K^k did not copatch. When H-2K^k was patched with antibody, FITC-G did not copatch. These diffusion and patching measurements rule out the possibility that these proteins have either extensive oligomeric associations or strong specific pairwise associations.**

There is now much evidence that virus-specific cytotoxic T lymphocytes (CTL) are dually specific for virus and for self cell surface antigens encoded by the major histocompatibility complex (1). Effector lymphocytes sensitized against virus-infected cells of a given haplotype will lyse virus-infected cells of the same haplotype with much higher efficiency than virus-infected cells of a different haplotype. A similar restriction holds for the afferent immune response. Virus-specific H-2-restricted secondary elicitation of CTL has been demonstrated by using membrane fragments as well as reconstituted membranes (2–11). These studies show that both viral protein and H-2 antigen must be present in the same reconstituted membrane in order to elicit the cellular response.

Three models can be considered, based on alternative hypothetical structures for the T-cell receptor(s). In one model, a specific molecular complex between viral protein and transplantation antigen preexists in the target membrane before interaction with the T cell and is recognized by the T-cell receptor. In the second model, a close physical association of the two antigens is stabilized only during their interaction with the T-cell receptor. In the third model, no close physical or chemical association between viral protein and transplantation antigen exists prior to, or during, interaction with the T cell. (This is the two-receptor or "dual receptor" model.) The T-cell receptor(s) involved in the secondary elicitation of CTL is presumed to be on precytotoxic T cells or on T helper cells or on both.

One biophysical approach to this problem is to use fluorescently labeled membrane components so that the distribution, motion, and interaction of these components can be studied by using optical techniques. In the work described here, purified G protein (G) of vesicular stomatitis virus (VSV) and purified H-2K^k protein were fluorescently labeled and reconstituted into cell-sized liposomes by a new technique. These reconstituted membranes are recognized in an H-2-restricted manner by T lymphocytes. We have measured the lateral diffusion of fluorescently labeled H-2K^k and G in these liposomes by the technique of fluorescence recovery after photobleaching. The formation of large molecular aggregates of these proteins should affect their diffusion coefficients. In addition, antibody-mediated copatching experiments were carried out to probe for strong specific binding between these two membrane proteins.

MATERIALS AND METHODS

Materials. VSV was prepared as described (12). H-2K^k was purified according to the method of Herrmann and Mescher (13). G was purified according to the method of Miller *et al.* (14). Monoclonal anti-H2K^k antibody was obtained from culture supernatant of the 11-4.1 cell line provided by The Salk Institute (15). It was purified by affinity chromatography on a protein A-Sepharose column (Pharmacia) (16). Rabbit antisera to G was obtained as described by Hale *et al.* (17) and was purified by affinity chromatography on protein A-Sepharose. Alloantiserum to H-2K^k was obtained from H. O. McDevitt (Stanford University School of Medicine) and was used without further purification. BALB.K (*k,k*) mice were produced in the Bowman Gray School of Medicine breeding colony. Male AKR/J (*k,k*) retired breeders were purchased from The Jackson Laboratory. The cell lines of YAC (*k,d*) (a lymphoma of A/SN origin), P815 (*d,d*) (a mastocytoma of DBA/2 origin), RDM4 (*k,k*) (a murine lymphoma of AKR origin), and SP2/0 (*d,d*) (a myeloma of MOPC21 origin) were maintained in RPMI-1640 supplemented with 10% fetal calf serum (heat-inactivated, 56°C, 45 min), 0.03% glutamine, 2-mercaptoethanol (50 μM), and penicillin and streptomycin (Flow Laboratories, McLean, VA).

Liposomes. Liposomes were made of 75 mol % dimyristoylphosphatidylcholine and 25 mol % cholsterol and prepared as described (18). The liposomal suspension was centrifuged at 500 × *g* for 7 min. The liposome pellet was resuspended in phosphate-buffered saline (P_i/NaCl) to give a final lipid concentration of ≈10 mM. This procedure resulted in a loss of <10% of the original lipid as determined by incorporation of [^{14}C]dipalmitoylphosphatidylcholine. Diffusion measurements were carried out on liposomes ranging in size from 10 to 50 μm.

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.

Abbreviations: CTL, cytotoxic T lymphocytes; P_i/NaCl, phosphate-buffered saline; FITC, fluorescein isothiocyanate; (*k,k*), the *H-2K* and *H-2D* alleles; VSV, vesicular stomatitis virus; G, G protein of VSV.

† Present address: Abbott Laboratories, North Chicago, IL 60064.
¶ To whom reprint requests should be addressed.

Protein Fluoresceination. Fluorescein isothiocyanate (FITC) (0.5 mg/ml) was added to a detergent solution of H-2K^k or G at pH 8.3 and the mixture was stirred at 4°C overnight. The labeled protein was purified by Sephadex G-25 gel chromatography followed by nondenaturing deoxycholate/polyacrylamide gel electrophoresis. The purified conjugated protein was found to be free of any detectable unconjugated fluorescein by $NaDodSO_4$/polyacrylamide gel electrophoresis (19). It was possible to detect fluorescent material with high sensitivity by examining the gel in an expanded laser beam (488 nm). The fluorescein-to-protein ratios of the purified conjugates were determined by measuring the protein concentration by the Lowry method (20) and the fluorescein concentration by absorbance at 495 nm ($\varepsilon = 7.4 \times 10^4$ 1/mol·cm). The ratios were 3 for FITC-H-2K^k and 4 for FITC-G.

Reconstitution. Reconstitution of membrane proteins was achieved by adding a detergent solution of the proteins to preformed liposomes. The relative volumes of detergent solution and liposomal suspension were chosen to avoid dissolution of the liposomes. This method was found to give good results for 0.2–0.5% deoxycholate or 30 mM octyl glucoside in either Tris-buffered saline (pH 8.0) or P_i/NaCl. In a typical experiment, 40 μl of protein solution (100–300 mg/ml in 0.5% deoxycholate/140 mM NaCl/10 mM Tris, pH 8.0) was added to 400 μl of the 10 mM liposome suspension in P_i/NaCl. The resulting solution was immediately dialyzed against 2 liters of P_i/NaCl plus 0.02% azide for at least 48 hr, with changes of buffer at approximately 12-hr intervals. Residual detergent was 0.1 mol % as monitored by using [^{3}H]deoxycholate. When more than one type of protein was added to a single set of liposomes, the proteins in detergent solution were mixed prior to addition of them to liposomes. Diffusion of fluoresceinated proteins in liposomes was measured as described (21). Bleach times of the order of 20 msec were used with a laser power density of $\approx 10^4$ W/cm^2 at 488 nm.

Patching Experiments. For experiments on liposomes with antibodies, liposomes reconstituted as described above were pelleted and resuspended in cell buffer [CB; 2.0 mM $CaCl_2$/1.5 mM $MgCl_2$/5.4 mM KCl/1 mM Na_2HPO_4/5.6 mM glucose/120 mM NaCl/0.2% bovine serum albumin (Calbiochem, fatty acid poor)/25 mM Hepes, pH 7.4]. All antibodies were centrifuged for 15 min at 18 psi in a Beckman Airfuge prior to use. Twenty microliters of liposomes (10 mM lipid) was incubated with 10 μl of antibody (1 mg/ml) on ice for 30 min. The liposomes were then washed twice in cold CB and resuspended in 10 μl of second-step reagent. The liposomes were incubated for 30 min on ice, washed three times with cold CB, resuspended in CB, and observed by visible and fluorescence microscopy using laser excitation at 488 nm for fluorescein and at 514 nm for rhodamine. Photomicrographs were taken on Kodak 2475 recording film at ASA 1600.

Generation of Effector Cells. Mice were primed by an intravenous injection of 5×10^5 plaque-forming units of VSV in 20% sucrose. Primed spleen cells (8.0×10^6 per spleen) were removed from these mice 4–6 wk later, cleared of erythrocytes, and cultured with varying amounts of liposomes or cells in 2.0 ml of supplemented RPMI-1640 in 1.7 × 1.6 cm wells (Linbro) in an atmosphere of 6% CO_2/94% air. After 5 days in culture, the cells were tested for their ability to lyse ^{51}Cr-labeled target cells.

Target Cells. Target cells were prepared by infecting 4.0×10^6 P815 or YAC with VSV for 2 hr at a multiplicity of infection of 25–50 at 37°C in an atmosphere of 6% CO_2/94% air. The cells were then incubated with 200 μCi (1 Ci = 3.7×10^{10} becquerels) of $Na_2{}^{51}CrO_4$ (New England Nuclear) in 0.5 ml of supplemented RPMI-1640 for 1.5 hr.

Cytotoxicity Assays. Cytotoxicity assays were carried out as described by Hale (22). Percentage specific release was calculated as $100 \times (E-C)/(1-C)$, in which E is the fraction of ^{51}Cr released by antigen-stimulated effector cells and C is the fraction of ^{51}Cr released by a mock-stimulated effector population.

RESULTS

Secondary Elicitation of CTL. The response to liposomes containing FITC-G and H-2K^k or FITC-H-2K^k and G was comparable to the response to liposomes containing G and H-2K^k (Fig. 1). Liposomes containing no protein did not stimulate CTL. Characterization of the effectors has demonstrated that they lyse only H-2K^k cells (YAC) infected with VSV (55% specific lysis) and not H-2K^d cells (P815) infected with VSV (5.1% specific lysis) or uninfected YAC cells (−7.7% specific lysis). These values were obtained with the highest liposome stimulator concentration ($\approx$2 μg of total protein per μmol of lipid; 1:2 mol ratio for H-2K^k/G). The biological activity of these fluorescent molecules renders meaningful the diffusion and copatching experiments described below.

Lateral Diffusion. Lateral diffusion measurements were made on four different sets of samples. Liposomes contained (*i*) FITC-H-2K^k; (*ii*) FITC-H-2K^k and G in equimolar amounts; (*iii*) FITC-G; or (*iv*) FITC-G and H-2K^k in equimolar amounts. The rate of lateral diffusion of FITC-H-2K^k was not influenced by the presence of G in the same membrane (Table 1). Similarly, the presence of H-2K^k did not influence the rate of diffusion of FITC-G. The recoveries we obtained consistently ranged from 30% to 40% and are comparable to recovery of a freely diffusing lipid probe measured under similar conditions (data not shown).

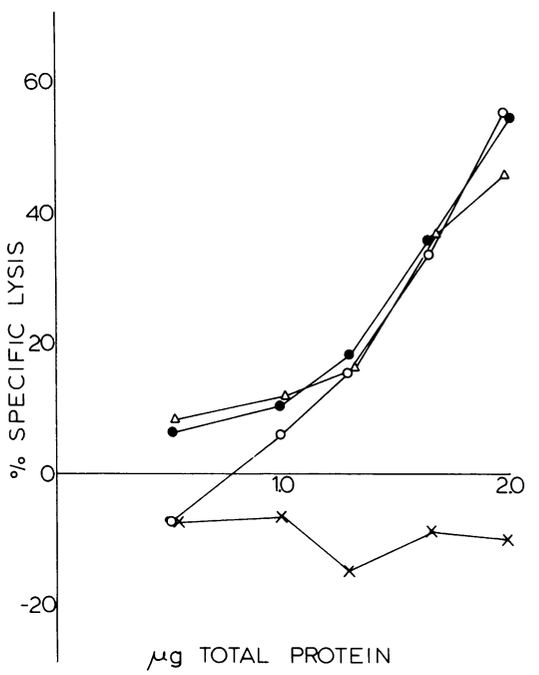

FIG. 1. Elicitation of anti-VSV CTL by G-H-2K^k liposomes containing FITC derivatives of G and H-2K^k proteins. BALB.K spleen cells primed 4 wk earlier with Indiana VSV (5.0×10^5 pfu per animal; intravenous injection) were incubated with liposomes (H-2K^k, 1 μg; G, 2 μg; total lipid, 1.5 μmol) at the concentrations of total liposomal proteins indicated. Four types of liposomes were used: G-H-2K^k liposomes (○), FITC-G-H-2K^k liposomes (●), G-FITC-H-2K^k liposomes (△), and no proteins (×) (see *text* for characterization of liposomes). After 5 days of culture the resultant anti-VSV-CTL were tested for cytolytic activity on ^{51}Cr-labeled Indiana VSV-infected YAC cells at an effector-to-target ratio of 50:1.

Table 1. Lateral diffusion

Protein	Temp., °C	D, $cm^2 \times 10^8/sec$*	% recovery†
FITC-G	37	1.14 ± 0.14	32 ± 3
FITC-G/H-2K^k	37	1.20 ± 0.16	36 ± 4
FITC-H-2K^k	37	1.13 ± 0.15	—
FITC-H-2K^k/G	37	1.07 ± 0.11	38 ± 3
FITC-G	25	0.45 ± 0.05	30 ± 4
FITC-G/H-2K^k	25	0.44 ± 0.06	32 ± 2
FITC-H-2K^k	25	0.36 ± 0.04	30 ± 2
FITC-H-2K^k/G	25	0.52 ± 0.06	33 ± 2

* Shown as mean ± SD.

† The theoretical maximal recovery is 50% and is determined by the structure of the Ronchi ruling used to obtain the bleach pattern (50% transparent). Typical recoveries for freely diffusing molecules in liposomes are <50% because of various optical effects (see *text*).

Copatching. We found that both nonfluorescent and fluorescent proteins could be patched by using double-antibody methods. The patching was monitored both by the fluorescence of the rhodamine-conjugated second-step antibody and by the fluorescence of the fluorescein-conjugated protein itself. When G was patched by using a rhodamine-conjugated second-step antibody, no copatching of FITC-H-2K^k was observed. When H-2K^k was patched by using a rhodamine-conjugated second-step antibody, no copatching of FITC-G was observed. Representative photomicrographs are shown in Fig. 2.

Reconstitution. The fraction of added protein that bound to liposomes was measured by two methods. In the first method, FITC-protein in detergent solution was added to liposomes, and the liposomes were immediately centrifuged at 1000 × g for 4 min. The fluorescence intensity of the supernatant was measured and compared to the initial fluorescence intensity of the protein solution before addition to liposomes. The liposomes were resuspended in the supernatant and dialyzed for ≈16 hr against P_i/NaCl. At the end of this period, the liposomes were again centrifuged and the fluorescence intensity of the supernatant was measured. We found that ≈40% of the added protein associated with the liposomes within 4 min and that complete association was achieved after 16 hr in that no detectable fluorescence remained in the supernatant.

In the second method, ^{125}I-labeled H-2K^k (^{125}I-H-2K^k) was used as a tracer (23). It was found that 90% of the radioactivity was associated with the liposomes after extensive dialysis. A Pronase digestion experiment was undertaken with reconstituted, dialyzed liposomes to determine the fraction of liposome-associated protein that was present in the outer bilayer of the multilamellar liposomes. The procedure of Curman *et al.* (24) was followed and it was found that 55–60% of the radioactivity was released by Pronase cleavage. This result was obtained for both ^{125}I-H-2K^k and ^{125}I-G. This is a lower limit on the fraction of protein in the outer bilayer of liposomes because the cleavage may not have been 100% efficient and the protein may also have been labeled at sites that were inaccessible to release by the enzyme. Less than 5% of the radioactivity was released from liposomes when no Pronase was added. The nearly complete patching of the fluorescein-conjugated protein reconstituted into liposomes (Fig. 2) is further evidence that these proteins are in the outer bilayer and probably in the proper orientation.

Antibody Binding. The procedure of Tsu and Herzenberg (25) to measure antibody binding was used with an ^{125}I-labeled protein A second-step reagent. In each well was placed either 4×10^5 cells or 20 μl of 40 mM liposomes. Each well was estimated to contain approximately the same amount of assayed protein. The results of antibody binding experiments are shown in Table 2. Both reconstituted G and FITC-G specifically bound rabbit anti-G antibodies. Reconstituted H-2K^k in liposomes specifically bound both monoclonal anti-H-2K^k from cell line 11-4.1 and alloantiserum against H-2K^k. FITC-H-2K^k did not bind the monoclonal antibody, and alloantiserum against H-2K^k bound FITC-H-2K^k only 25–30% as well as nonfluorescent H-2K^k.

Table 2. Antibody binding at various antibody dilutions

	11-4.1 monoclonal anti-H-2K^k		Alloantiserum anti-H-2K^k		Rabbit anti-G	
	1:2	1:20	1:2	1:20	1:2	1:20
RDM4	7363	7247	6696	3043	422	437
YAC	1201	511	883	573	232	104
H-2K^k in liposomes	8251	8627	6841	2451	278	140
FITC-H-2K^k in liposomes	965	476	1649	774	265	156
G in liposomes	872	216	196	743	5828	1630
FITC-G in liposomes	761	280	299	164	5320	1842
Liposomes	720	190	157	84	144	124

Numbers are cpm from ^{125}I-labeled protein A second-step reagent bound to first-step antibody.

DISCUSSION

From the studies described here we draw two conclusions.

(*i*) A fluorescein-conjugated murine transplantation antigen (H-2K^k) and a fluorescein-conjugated virus protein (VSV G) in reconstituted lipid membranes retain their capacity to elicit an *H-2*-restricted virus protein-specific secondary CTL response (Fig. 1). The CTL response to fluorescein-conjugated antigens is quantitatively the same as the response to nonfluorescent antigens. Experiments in which the two-dimensional concentrations of H-2K^k and FITC-H-2K^k were varied (and total protein held constant) showed equivalent CTL elicitations, demonstrating that the cellular response to these liposomes is not due to a small fraction of nonfluorescent protein in the fluorescent liposomes. This conclusion is particularly interesting in that the FITC-H-2K^k does not bind 11-4.1 antibody, and it binds the alloantiserum only weakly.

Our results conflict with the report by Rosenthal and Zinkernagel (26) that VSV-specific *H-2*-restricted recognition does not occur for *H-2K^k* and *H-2D^k* allotypes. However, this restriction has been reported earlier by Hale *et al.* (17).

(*ii*) Lateral diffusion and copatching results show no detectable association between H-2K^k and G. Both the rate of lateral diffusion and the recovery amplitude of each protein was unaffected by the presence of the other (Table 1). The rate of lateral diffusion of these proteins is comparable to rates observed for other membrane proteins in similar lipid mixtures (27–28). The recovery amplitudes are similar to those observed for freely diffusing lipid probes in the same liposomes (data not shown). The deviations of these numbers from 50% is due to optical effects such as limited depth of field and diffraction. The observed variation in recovery amplitude (30–40%) is thought to be due to optical effects related to variation in liposome size, objective lens focusing, and bleach stripe position.

These lateral diffusion measurements cannot rule out the formation of small protein complexes such as those found in immunoprecipitation experiments (22). Saffman–Delbrück theory (29) predicts a weak dependence of lateral diffusion on protein size. Using this theory we estimate that a 30% difference in lat-

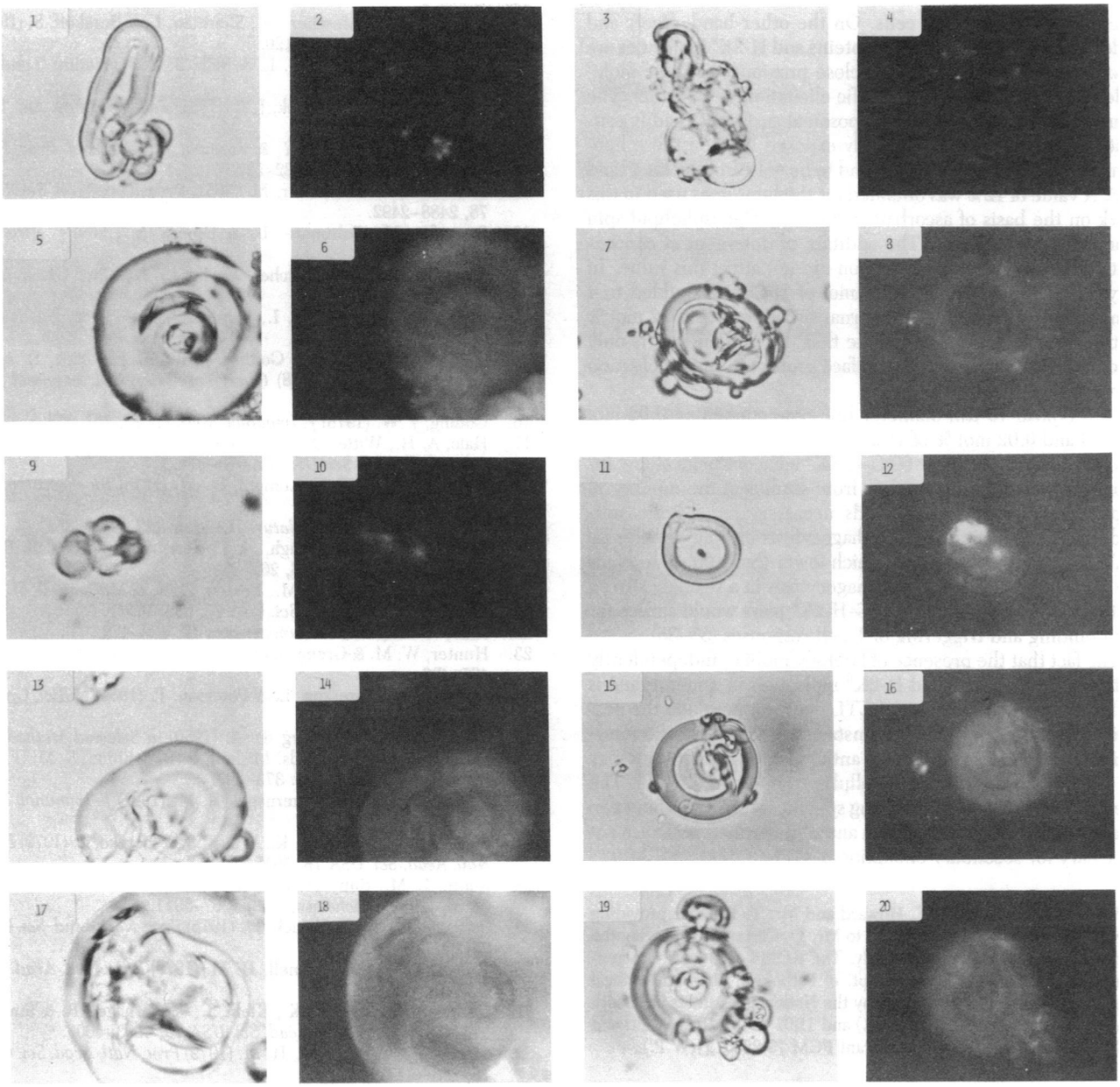

FIG. 2. Transmitted light (odd numbers) and fluorescence (even numbers) photomicrographs of liposomes. Labeled and unlabeled H-2K^k or G was reconstituted into liposomes (≈2 μg of total protein per μmol of lipid, H-2K^k/G, 1:2 mol ratio) and, in some cases, labeled with antibodies as described in text. Transmitted light and fluorescence photomicrographs of the same liposomes are shown for each case. (*1*, *2*) Unlabeled H-2K^k patched with unlabeled 11-4.1 antibody (first-step) and rhodamine-conjugated F(ab')$_2$ rabbit anti-mouse antibody (Cappel, Cochranville, PA) (second-step, abbreviated RFRAM henceforth); (*3*, *4*) unlabeled G patched with rabbit anti-G and rhodamine-conjugated F(ab')$_2$ goat anti-rabbit (Cappel) (abbreviated RFGAR henceforth); (*5*, *6*) FITC H-2K^k; (*7*, *8*) FITC-G; (*9*, *10*) FITC-H-2K^k patched with alloantiserum against H-2K^k and RFRAM (fluorescein fluorescence); (*11*, *12*) FITC-G patched with rabbit anti-G and RFGAR (fluorescein fluorescence); (*13*, *14*) FITC-H-2K^k and unlabeled G; (*15*, *16*) FITC-G and unlabeled H-2K^k; (*17*, *18*) FITC-H-2K^k and unlabeled G (fluorescein fluorescence) with the G protein patched with rabbit anti-G and RFGAR; (*19*, *20*) FITC-G and unlabeled H-2K^k (fluorescein fluorescence) with the H-2K^k patched with 11-4.1 antibody and RFRAM. (The field of each photomicrograph is ≈40 × 50 μm except for *17* and *18* which are magnified by a factor of less than 2.)

eral diffusion coefficient corresponds to approximately a 7-fold change in the radius of the protein in the bilayer. Similarly, a 20% difference corresponds to a 4-fold change in protein radius. This theory has not yet been tested critically. It does not take into account probable distortions of lipid structure due to integral membrane proteins and the effect this may have on the relationship between size and diffusion (30).

The lack of copatching of the two proteins also argues against extensive oligomeric protein–protein association or energetically strong pairwise association. The degree of protein–protein association that copatching experiments can detect is a complex quantitative problem. However, it is likely that such copatching experiments are quite sensitive to strong pairwise protein–protein associations. Copatching of G and H-2K^b on VSV-infected cells has been studied by Geiger *et al.* (31). Although their results are quite difficult to interpret in molecular terms, they did suggest an absence of a strong interaction between G and H-2K^b molecules before antibody binding.

A strong specific interaction between viral proteins and H-2K^k molecules in a reconstituted membrane would provide strong support for any theory that required close proximity of these two molecules in the target membrane during specific

elicitation of cytotoxic T cells. On the other hand, freely and independently diffusing viral proteins and H-2K^k molecules are also consistent with a possible close proximity of these molecules as a prerequisite for specific elicitation of CTL (32). The density of H-2K^k or G on the liposomal surface is readily estimated. The amount of externally exposed lipid on these liposomal preparations has been found to be ≈10% of the total lipid (18). A value of 12% was obtained for the liposomes used in this work on the basis of ascorbate reduction of phospholipid spin labels (data not shown). The addition of detergent at concentrations used in the reconstitution did not affect this value. In a typical reconstitution, 10^{-4} μmol of H-2K^k was added to 4 μmol of lipid. Because the external surface contains 12 mol % of the total lipid, and we assume that the protein inserts only into the external surface, the surface protein-to-lipid mole ratio is ≈0.02 mol %.

In a typical 10-μm-diameter liposome containing 0.02 mol % of G and 0.02 mol % of H-2K^k in the outer bilayer, one can calculate that there are ≈60 G-H-2K^k adjacent pairs at any instant of time. If we extrapolate from studies of the number of hapten–antibody–receptor bonds necessary for specific antibody-dependent binding and phagocytosis of lipid vesicles by RAW264 macrophages (with which fewer than 100 such bonds are sufficient for binding and phagocytosis in a strongly stirred system), it is plausible that 60 G-H-2K^k pairs would suffice for both binding and triggering of T-cell responses (33–35).

The fact that the presence of laterally mobile, independently diffusing viral protein and H-2K^k molecules in a membrane is sufficient for the elicitation of CTL does not preclude the possibility that, under some circumstances, strong specific interactions between transplantation antigens and viral proteins may be observed and also lead to cellular stimulation (36–38). The present work suggests that strong specific interactions between viral protein and transplantation antigens in membranes are not necessary for secondary elicitation of CTL.

We are indebted to Dr. F. Howard and Mr. T. Frey for providing us with purified VSV G protein and to Mr. D. Carter for supplying the purified 11-4.1 monoclonal antibody. The RDM4 cells were the generous gift of Dr. M. Mescher (Dept. of Pathology, Harvard Medical School). This work was supported by the National Institutes of Health Grants 5R01 AI 13587 (H.M.Mc.C.) and 1R01 AI 16810 (J.W.P.) and by National Science Foundation Grant PCM 79-22937 (J.W.P.).

1. Zinkernagel, R. M. & Doherty, P. C. (1979) *Adv. Immunol.* **27**, 52–177.
2. Loh, D., Ross, A. H., Hale, A. H., Baltimore, D. & Eisen, H. N. (1979) *J. Exp. Med.* **150**, 1067–1074.
3. Ciavarra, R. P., Kang, C. Y. & Forman, J. (1980) *J. Immunol.* **125**, 336–343.
4. Mescher, M., Sherman, L., Lemonnier, F. & Burakoff, S. (1978) *J. Exp. Med.* **147**, 946–951.
5. Finberg, R., Mescher, M. & Burakoff, S. (1978) *J. Exp. Med.* **148**, 1620–1627.
6. Senik, A. & Neauport-Sautes, C. (1979) *J. Immunol.* **122**, 1461–1467.
7. Lemonnier, F., Mescher, M., Sherman, L. & Burakoff, S. (1978) *J. Immunol.* **120**, 1114–1120.
8. Mescher, M., Jose, M. J. L. & Balk, S. (1981) *Nature (London)* **289**, 139–144.
9. Doherty, P. C. & Bennick, J. R. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 218–221.
10. Ciavarra, R., Kang, C. Y. & Forman, J. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 222–227.
11. Herrmann, S. & Mescher, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2488–2492.
12. Stampfer, M., Baltimore, D. & Huang, A. (1969) *J. Virol.* **4**, 154–161.
13. Herrmann, S. H. & Mescher, M. F. (1979) *J. Biol. Chem.* **254**, 8713–8716.
14. Miller, D. K., Feuer, B. I., Vanderoef, R. & Lenard, J. (1980) *J. Cell. Biol.* **84**, 421–429.
15. Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A. & Herzenberg, L. A. (1978) *Curr. Top. Microbiol. Immunol.* **81**, 115–129.
16. Goding, J. W. (1978) *J. Immunol. Methods* **20**, 241–253.
17. Hale, A. H., Witte, O. N., Baltimore, D. & Eisen, H. N. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 970–974.
18. Schwartz, M. A. & McConnell, H. M. (1978) *Biochemistry* **17**, 837–841.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
21. Petty, H. R., Smith, L. M., Fearon, D. T. & McConnell, H. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6587–6591.
22. Hale, A. H. (1980) *Immunogenetics* **10**, 469–479.
23. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496.
24. Curman, B., Klareskog, L. & Peterson, P. (1980) *J. Biol. Chem.* **255**, 7820–7826.
25. Tsu, T. T. & Herzenberg, L. A. (1980) in *Selected Methods in Cellular Immunology*, eds. Mishell, B. B. & Shiigi, S. M. (Freeman, San Francisco), pp. 373–397.
26. Rosenthal, K. L. & Zinkernagel, R. M. (1981) *J. Immunol.* **126**, 446–451.
27. Vaz, W. L. C., Jacobson, K., Wu, E. S. & Derzko, Z. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5645–5649.
28. Smith, L. M., Rubenstein, J. L. R., Parce, J. W. & McConnell, H. M. (1980) *Biochemistry* **19**, 5907–5911.
29. Saffman, P. G. & Delbruck, M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3111–3113.
30. Owicki, J. C. & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4750–4754.
31. Geiger, B., Rosenthal, K., Klein, J., Zinkernagel, R. & Singer, S. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4603–4607.
32. Maeda, T. & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1537–1541.
33. Hafeman, D. G., Lewis, J. T. & McConnell, H. M. (1980) *Biochemistry* **19**, 5387–5394.
34. Hafeman, D. G., Parce, J. W. & McConnell, H. M. (1979) *Biochem. Biophys. Res. Commun.* **86**, 522–528, and [correction (1980) **94**, 735].
35. Lewis, J. T., Hafeman, D. H. & McConnell, H. M. (1980) in *Liposomes and Immunobiology*, eds. Tom, B. H. & Six, H. R. (Elsevier, New York), pp. 179–191.
36. Hecht, T. T. & Summers, D. F. (1972) *J. Virol.* **10**, 578–585.
37. Hecht, T. T. & Summers, D. F. (1976) *J. Virol.* **19**, 833–845.
38. Bubbers, J. E., Chen, S. & Lilly, F. (1978) *J. Exp. Med.* **147**, 340–351.