STUDIES ON THE IMMUNE RESPONSE TO A CHARACTERIZED ANTIGENIC DETERMINANT OF THE TOBACCO MOSAIC VIRUS PROTEIN*

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The recent use of chemically defined polypeptides to investigate immunological phenomena has resulted in a wealth of information regarding the chemical and biological basis of antigenicity, immunogenicity, skin reactivity, and cell-antigen interactions. Information obtained from chemically defined systems is more meaningful and comprehensive than that obtained from the chemically ill-defined systems previously used, in that these systems lend themselves more readily to specific experimental manipulation. In these respects peptides and proteins of known amino acid sequence and/or stucture offer excellent immunochemical tools. Studies utilizing these systems have been recently reviewed (1–4).

Immunochemical studies on one well-characterized protein, namely the tobacco mosaic virus protein (TMVP), which were conducted during the past several years, resulted in the isolation, characterization, and synthesis of a major antigenic determinant of the protein, and in the understanding of several of the principles underlying the binding of the determinant with antibodies. An eicosapeptide, representing residues 93–112 of the protein and having the amino acid sequence Ileu-Ileu-Glu-Val-Glu-$AspNH_2$-$GluNH_2$-Ala-$AspNH_2$-Pro-Thr-Thr-Ala-Glu-Thr-Leu-Asp-Ala-Thr-Arg, was found to bind specifically with antibodies produced by rabbits in response to immunization with TMVP (5, 6). Subsequent work demonstrated that anti-TMVP antibodies also bound with the C-terminal decapeptide portion of the eicosapeptide (7). Although antibodies capable of binding with the C-terminal di-, tri-, or tetrapeptide could not be demonstrated, some rabbits produced antibodies which bound with the C-terminal pentapeptide, while others produced antibodies which required the C-terminal hexa- or heptapeptide for demonstrable binding (8, 9). However, antibodies produced by all

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rabbits tested were shown to bind specifically with the *N*-octanoylated C-terminal tripeptide, namely with *N*-octanoyl-Ala-Thr-Arg (10). Similarly, studies with anti-TMVP antibodies produced by guinea pigs demonstrated specific binding with the eicosapeptide and its C-terminal decapeptide. Binding between the C-terminal pentapeptide and pooled globulins obtained from TMVP-sensitized guinea pigs was also shown.[1]

The work on the immunochemistry of the TMVP peptides was hitherto aimed primarily at elucidating the principles underlying the binding of the determinant with antibodies to the protein. In view of the findings described, it was apparent that the use of this well-characterized system would serve as a powerful tool for the investigation of cellular immune responses as well. The present communication reports results of experiments dealing with the immunogenicity of the peptides, with their ability to elicit skin reactions in TMVP-sensitized animals, with their capacity to stimulate DNA synthesis in lymphocytes derived from TMVP-sensitized animals, and with their ability to inhibit the migration of peritoneal exudate cells derived from such animals.

Materials and Methods

Tobacco Mosaic Virus Protein (TMVP).—The protein was generously supplied by Dr. C. A. Knight, Virus Laboratory, University of California, Berkeley, Calif. TMVP was prepared from the virus as described by Fraenkel-Conrat (11).

TMVP Peptides[2].—The eicosapeptide representing residues 93–112 of TMVP was obtained from a tryptic digest of the protein as previously described (12, 5). All other peptides were synthesized by the Merrifield solid phase peptide synthesis method (13) as described (7, 8, 14). The deca- and pentapeptides were purified and analyzed as previously described (7, 8). Results of amino acid analyses are given in Table I.

$(Lys)_n$ pentapeptides were synthesized by the sequential coupling of lysine residues onto the pentapeptide-resin using t-Boc-*N*-ϵ carbobenzyloxy-L-lysine (obtained from Fox Chemical Co., Los Angeles, Calif.). After 6 and 10 lysine couplings, the cleaved peptides contained an average of only 4.26 and 7.18 lysine residues, respectively (Table I). Electrophoresis of these crude peptides performed at pH 6.4 revealed the presence of several lysylpeptides, showing that these peptides were heterogeneous with respect to lysine residues. For convenience, the synthesized peptides are designated as $(Lys)_4$ pentapeptide and $(Lys)_7$ pentapeptide.

The *N*-acyl-Ala-Thr-Arg derivatives were prepared by solid phase synthesis in a manner similar to the preparation of *N*-octanoyl-Ala-Thr-Arg previously described (15). Butyric, hexanoic, octanoic, and decanoic acids (99–100% pure, obtained from Sigma Chemical Co., St. Louis, Mo.) were coupled to the tripeptide-resin using 4 moles fatty acid and 4 moles of dicyclohexylcarbodiimide per mole of peptide. The acylpeptides were cleaved from the resin with HBr-trifluoroacetic acid, dissolved in a mixture of methanol:water:acetic acid (50:40:10), and reduced overnight by catalytic hydrogenation at atmospheric pressure as described (14). The acylpeptides were electrophoresed at pH 6.4. Ninhydrin and Sakaguchi staining

[1] Benjamini, E. Unpublished results.

[2] The approximate mol wts of the antigens utilized in these studies are as follows: TMVP, 17,000; eicosapeptide, 2184; decapeptide, 1077; pentapeptide, 574; $(Lys)_7$ pentapeptide, 1470; $(Lys)_4$ pentapeptide, 1086; butyryl-tripeptide, 416; hexanoyl-tripeptide, 444; octanoyl-tripeptide, 472; decanoyl-tripeptide, 500; BSA, 66,000.

revealed that Sakaguchi positive peptides were ninhydrin negative and neutral, indicating complete N acylation. The amino acid analyses of the *N*-acyl-Ala-Thr-Arg peptides are given in Table I.

Radioiodinated TMVP.—Approximately 1 mg protein was iodinated with 2 mCi (^{125}I)-NaI in 0.1 N NaOH (obtained from New England Nuclear Corp., Boston, Mass.) according to the procedure described by Greenwood et al. (16). The (^{125}I) TMVP was recovered after passage through a G-25 Sephadex column (1.5 × 75 cm) equilibrated and eluted with 0.1 M $NaHCO_3$. The radioiodinated protein had a specific activity of 1×10^6 cpm/μg. For antibody assay, it was diluted with 100-fold excess of noniodinated TMVP in 0.1 M $NaHCO_3$ containing 1% bovine serum albumin (BSA) obtain from Pentax Inc., Kankakee, Ill.

Immunization.—Male and female guinea pigs weighing approximately 350 g were immunized with TMVP, with peptides, or with BSA in borate-buffered saline, pH 8, emulsified

TABLE I

*Amino Acid Analysis of TMVP Peptides**

Peptide	Molar ratio									
	Lys	Arg	Asp	Thr	Glu	Pro	Ala	Val	Ileu	Leu
Ileu-Ileu-Glu-Val-Glu-$AspNH_2$-$GluNH_2$-Ala-$AspNH_2$-Pro-Thr-Thr-Ala-Glu-Thr-Leu-Asp-Ala-Thr-Arg (eicosapeptide)		1.04	2.99	3.78	4.03	1.10	2.96	1.04	0.88	1.01
Thr-Thr-Ala-Glu-Thr-Leu-Asp-Ala-Thr-Arg (decapeptide)		0.90	1.03	3.93	1.06		2.11			1.00
Leu-Asp-Ala-Thr-Arg (pentapeptide)		1.02	0.98	1.00			1.00			0.99
$(Lys)_4$-pentapeptide	4.26	0.88	0.96	0.97			1.08			1.00
$(Lys)_7$-pentapeptide	7.18	0.92	0.99	1.02			1.02			0.97
Butyryl-Ala-Thr-Arg		1.16		N.D.			0.84			
Hexanoyl-Ala-Thr-Arg		0.95		1.01			1.03			
Octanoyl-Ala-Thr-Arg		0.93		1.05			1.02			
Decanoyl-Ala-Thr-Arg		0.96		1.00			1.04			

*The peptides were hydrolyzed under open reflux at 110°C for 24 hr and quantitative amino acid analyses performed using the Spinco Model 120B amino acid analyzer.

with an equal volume of Freund's complete adjuvant containing *Mycobacterium butyricum* (Difco Laboratories, Inc., Detroit, Mich.). Injections consisted of 0.1 ml administered intradermally into each footpad, 0.3 ml administered intramuscularly, and 0.3 ml given subcutaneously. Animals were sensitized to TMVP with a total dose of 0.15 μmole (2.5 mg). Animals sensitized with BSA were given a total antigen dose of 0.038 μmole (2.5 mg). Doses of the peptides injected ranged from 0.05 μmole to 7.5 μmole. The animals were bled for antibody studies 1 month after the immunizations and were skin tested immediately thereafter. For studies of lymphocyte stimulation and capillary migration, animals were used 1–2 months after immunization.

Skin Tests.—Skin tests were performed on a shaved area of the flank by intradermal injections of 0.1 ml of antigen in borate-buffered saline, pH 8. Skin reactions were recorded at the end of 1 hr (immediate reactions) and 24 hr (delayed reactions) after the challenge. Reactions were considered positive if they consisted of erythema and induration of 5 mm or greater.

Antibody Determination.—The test antiserum was reacted with (^{125}I)TMVP and the complexes precipitated overnight in the cold by rabbit anti-guinea pig serum. Radioactivity was

measured in the supernatant using the Model 1810 radiation analyzer and Model D5 (203V) well scintillation detector (Nuclear-Chicago Corp., Des Plaines, Ill.).

Lymphocyte Stimulation.—Splenic lymphocytes were cultured according to the method of Dutton and Eady (17); 10×10^6 cells were cultured in 4 ml of Eagle's minimal essential medium for suspended cultures (Spinner's medium, obtained from Grand Island Biological Co., Grand Island, N. Y.), containing 100 units of penicillin and 100 μg of streptomycin per ml, 1% L-glutamine, and 15% calf serum. All cultures were prepared in duplicate or triplicate. Antigen was added in 0.1 ml saline at the beginning of the culture, and the mixtures were incubated at 37°C in an atmosphere of 5% CO_2 and 95% air for 2 days, at the end of which period 0.1 μCi of 2 ^{14}C-thymidine (specific activity, 59.2 mCi/mole, obtained from New England Nuclear Corp.) was introduced into each culture tube. The cells were harvested in the cold 24 hr later by serially suspending them once in saline, twice in 5% trichloroacetic acid, and once in methanol. The precipitate was dissolved by incubation in 1 ml of Hyamine at 56°C for 1 hr, transferred to counting vials, and prepared for scintillation counting by adding 12 ml of Omnifluor (New England Nuclear Corp.) in toluene (4 g Omnifluor in 1 liter toluene). The radioactivity was measured on the Nuclear-Chicago Mark I liquid scintillation counter. The variation between replicate tubes was not more than ±10%.

Capillary Migration.—Capillary migration was performed as described by David et al. (18). Peritoneal exudates were induced in guinea pigs by the intraperitoneal injection of 30 ml light mineral oil (Marcol, Esso Humble Oil and Refining Co., Houston, Texas). 3 days later the cells were harvested by washing the peritoneal cavity with 200 ml of Hanks' solution. The packed cells were suspended in Spinner's medium, 7.5% by volume, and capillary tubes filled with the cell suspension. The tubes were sealed and spun in the cold for 5 min at 900 rpm. They were cut at the cell-fluid interface and placed in Mackaness-type culture chambers, two tubes per chamber. All chambers were prepared in duplicate. The chambers were filled with Spinner's medium containing 100 units of penicillin and 100 μg of streptomycin per ml, 1% L-glutamine, 15% normal guinea pig serum, and various concentrations of the test antigen. They were then sealed and incubated for 24 hr at 37°C. The cell image was projected and traced, and the area of migration measured using a planimeter. The areas of migration in duplicate test chambers (four tubes) were averaged, and the results were expressed as per cent of migration of peritoneal cells in the control chambers containing no antigen.

RESULTS

Immunogenicity.—Groups of guinea pigs were injected with 0.05, 0.5, or 5.0 μmoles of octanoyl tripeptide, with 0.05, 0.5, or 7.5 μmoles of pentapeptide, or with 0.05, 0.5, or 2.5 μmoles of decapeptide in complete Freund's adjuvant. These injections did not elicit skin sensitization, antibody formation, or lymphocyte sensitization.

Peptide-injected animals were skin tested with 6 mμmoles TMVP and did not demonstrate either immediate or delayed skin reactivity (Table II), whereas control animals immunized with 0.15 μmoles TMVP had positive immediate and delayed reactions to TMVP. Animals immunized with 0.038 μmoles BSA did not react to challenges given with TMVP, nor did the TMPV or peptide-injected animals react to challenges given with 1.5 mμmole BSA.

Individual sera obtained from the peptide-injected animals were titered with (^{125}I)TMVP. Results showed that antibodies capable of binding with TMVP were not produced by any of the animals. By comparison, TMVP-sensitized

animals produced antibodies capable of binding the antigen (Fig. 1). The specificity of this reaction was confirmed with (^{125}I)lysozyme.

Animals which were injected with each peptide and TMVP-sensitized animals which had not been used for skin tests were sacrificed. Their splenic

TABLE II

Skin Reactions to TMVP in Guinea Pigs Injected With TMVP or With Antigenic Peptides of TMVP

Antigen injected	Dose injected	Skin reactions	
		Immediate (1 hr)	Delayed (24 hr)
	μmole		
Decapeptide	0.05	0/3*	0/3
	0.5	0/4	0/4
	2.5	0/4	0/4
Pentapeptide	0.05	0/4	0/4
	0.5	0/3	0/3
	7.5	0/4	0/4
Octanoyl-tripeptide	0.05	0/4	0/4
	0.5	0/4	0/4
	5.0	0/12	0/12
TMVP	0.15	5/5	5/5

* Number of animals showing positive reactions/number of animals tested with 6 mμmole TMVP.

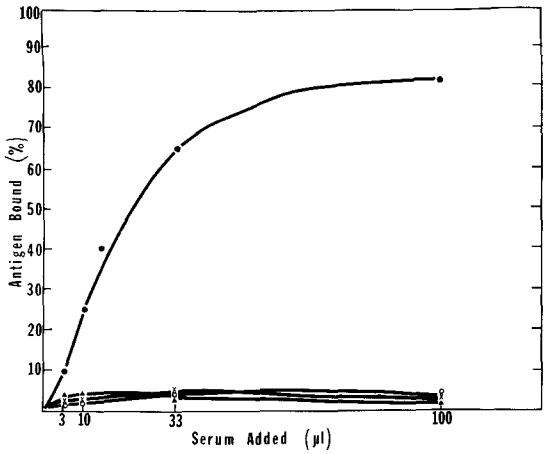

FIG. 1. Average binding of ^{125}I-TMVP by sera of guinea pigs injected with TMVP (●), with pentapeptide (○), with decapeptide (△) or with octanoyl-tripeptide (×). The amount of ^{125}I-TMVP added was 0.006 mμmoles.

lymphocytes were cultured with TMVP in a dose range of 0.75–750 μμmole/ml, and stimulation of DNA synthesis was measured by incorporation of (^{14}C)-thymidine. Whereas the TMVP stimulated thymidine uptake in lymphocytes

TABLE III

In Vitro ^{14}C-Thymidine Incorporation in Response to TMVP in Spleen Cell Cultures Derived From TMVP and Peptide Injected Guinea Pigs

Antigen injected	Dose injected	Stimulation*
	μmole	
Decapeptide	2.5	1.0 (2)‡
Pentapeptide	7.5	1.7 (1)
Octanoyl-tripeptide	5.0	1.0 (2)
TMVP	0.15	4.3 (2)

* Ratio of ^{14}C-thymidine incorporation in cultures with 750 μμmole/ml TMVP relative to incorporation in control cultures, from the same guinea pig, without TMVP.

‡ Parentheses indicate number of animals tested.

TABLE IV

Skin Reactions in Guinea Pigs Immunized with TMVP

Challenging antigen	Test dose	Skin reactions	
		Immediate (1 hr)	Delayed (24 hr)
	mμmole		
TMVP	6	6/9*	9/9
	0.6	5/9	6/9
$(Lys)_7$ pentapeptide	750	1/4	2/5
	75	2/9	1/9
$(Lys)_4$ pentapeptide	700	5/9	6/9
	70	0/9	1/9
Decapeptide	320	2/9	2/9
Pentapeptide	1500	3/9	2/4
	150	3/9	4/9
Hexanoyl-tripeptide	1000	0/9	3/9
Butyryl-tripeptide	1000	6/9	3/4
BSA	1.5	0/9	1/9
PPD	10 μg	0/9	9/9

* Number of animals showing positive reactions/number of animals tested.

derived from TMVP-sensitized animals, the lymphocytes derived from the animals injected with the peptides were not significantly stimulated (Table III).

Skin Reactions.—In order to determine the ability of the peptides to elicit skin reactions, guinea pigs were immunized with TMVP, and subsequently skin tested with TMVP and the TMVP peptides. Preliminary results indicated that low test doses of the peptides (in the range of 0.05 μmoles) did not elicit skin reactions. At higher concentrations, however, all of the peptides listed in

Table IV elicited specific delayed reactions in at least some of the animals (Table IV). Delayed reactions were always elicited by PPD, since all the animals were injected with Freund's complete adjuvant. In addition to the delayed

TABLE V

In Vitro ^{14}C-Thymidine Incorporation in Response to TMVP Peptides in Spleen Cell Cultures Derived from TMVP Sensitized Guinea Pigs

Antigen	Dose range	Average stimulation at highest concentration*
	mμmole/ml	
TMVP	0.00075–0.75	4.8 (7)‡
Pentapeptide	0.04–400	1.5 (2)
Decapeptide	1.6–160	0.84 (1)
Octanoyl-tripeptide	0.250–250	1.1 (3)
Eicosapeptide	1.7–250	1.2 (4)

* Ratio of ^{14}C-thymidine incorporation in cultures with antigen present relative to ^{14}C-thymidine incorporation in control cultures, from the same guinea pig, without antigen.

‡ Number of animals tested.

TABLE VI

Effect of TMVP and TMVP Peptides on the Migration of Peritoneal Exudate Cells Derived From Guinea Pigs Immunized With TMVP or Tuberculin

Antigen	Concentration in chamber	Migration*	
		TMVP sensitive cells	PPD sensitive cells
	μmole/ml	%	%
TMVP	0.006	58	107
TMVP	0.003	53	83
TMVP	0.0006	47	96
Pentapeptide	0.5	40	109
Decapeptide	1.0	37	138
$(Lys)_4$-pentapeptide	1.0	47	82
Butryl-tripeptide	1.0	50	87
Hexanoyl-tripeptide	1.0	45	111
Hexanoyl-tripeptide	0.5	69	136
Octanoyl-tripeptide	1.0	42	100
Octanoyl-tripeptide	0.5	84	87
Decanoyl-tripeptide	1.0	61	93
PPD	15 μg	14	29

* Numbers represent per cent migration in experimental chambers (compared to that in control chambers incubated without antigen).

reactions, all of these peptides, except the hexanoyl-tripeptide, elicited specific immediate reactions in some of the test animals. The specificity of the delayed and the immediate reactions was confirmed by the fact that guinea pigs sen-

sitized to BSA and challenged with the peptides did not demonstrate skin reactions when they were tested with the peptides in the same concentrations as those used to elicit reactions in TMVP-sensitized animals. Positive skin

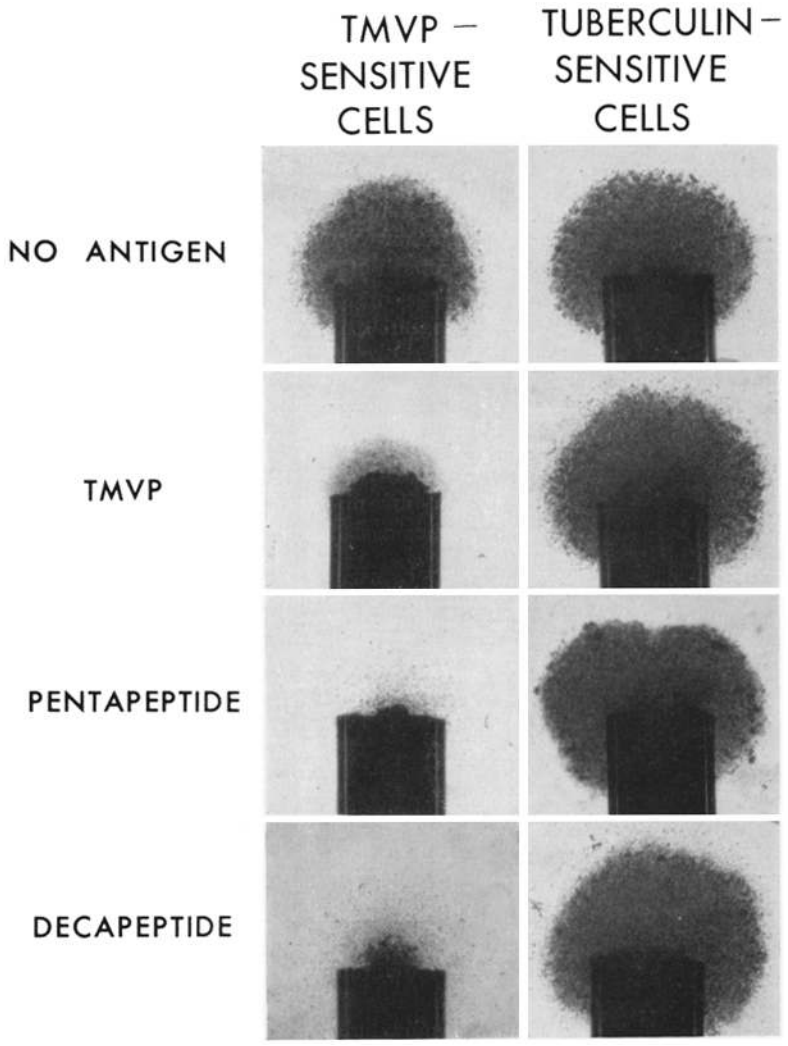

FIG. 2. Effect of TMVP and peptides on the migration of peritoneal exudate cells derived from guinea pigs immunized with TMVP or tuberculin.

tests were obtained with 1 μmole of the octanoyl- and decanoyl-tripeptide; however, these reactions were considered nonspecific since similar reactions were also elicited in BSA-sensitized guinea pigs.

Lymphocyte Stimulation.—Lymphocytes derived from TMVP-sensitized ani-

mals were cultured with TMVP or the TMVP peptides in order to determine whether or not the peptides could stimulate the incorporation of (^{14}C)thymidine by these cells. Both TMVP and the peptides were tested in a wide range of concentrations. The results show that whereas TMVP caused lymphocyte stimulation, the peptides did not significantly stimulate at any of the concen-

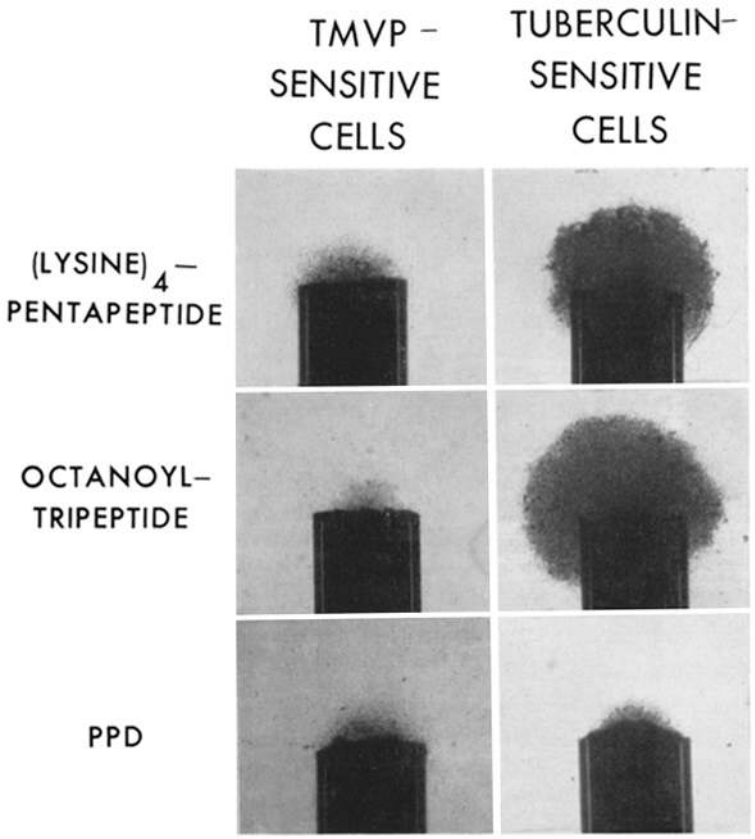

FIG. 3. Effect of TMVP peptides and PPD on the migration of peritoneal exudate cells derived from guinea pigs immunized with TMVP or tuberculin.

trations tested. Data for the stimulation in cultures containing the highest antigen concentration are given in Table V. The specificity of stimulation by TMVP was demonstrated by the fact that TMVP in the range of concentrations tested failed to stimulate splenic lymphocytes derived from BSA-sensitized guinea pigs, and by the fact that BSA, at a wide range of concentrations, did not stimulate TMVP-sensitized lymphocytes.

Inhibition of Capillary Migration.—Results given in Table VI show that the capillary migration of peritoneal exudate cells derived from TMVP-sensi-

tized guinea pigs was specifically inhibited by TMVP, by the pentapeptide, by the decapeptide, by the $(Lys)_4$ pentapeptide, and by the *N*-acyl-tripeptides. At the same concentrations, these antigens did not significantly inhibit the migration of the peritoneal exudate cells derived from tuberculin-sensitive animals. Results given are the averages of two experiments. As expected, PPD was inhibitory since both the TMVP and BSA were injected in Freund's complete adjuvant. Representative results of the inhibition of migration are illustrated in Figs. 2 and 3.

DISCUSSION

Data presented in this communication show that the antigenic peptides of TMVP (i.e., those capable of combining with anti-TMVP) are non immunogenic. This is evident from the findings that animals which were injected with the peptides in a wide range of doses failed to produce skin reactions to intradermal injections of TMVP, nor did they produce antibodies capable of reacting with (^{125}I)TMVP. Further, splenic lymphocytes derived from these animals could not be stimulated by TMVP.

It should be noted that the test animals were challenged with the whole antigen, TMVP, rather than with the injected peptides. Previous work on the binding of TMVP peptides with antibodies showed that the binding of the determinant with antibodies increases with increase in the length of peptide chain (9). It was therefore felt that the chances for the detection of an immune response to the peptides would be increased by using the whole antigen rather than the peptides for testing.

There is no clear cut minimum size requirement for a compound containing an antigenic determinant to be able to induce the immune response. It is generally accepted that antigens of relatively high molecular weight are usually immunogenic. Many compounds of low molecular weight are nonimmunogenic or are immunogenic only when coupled to a larger molecule. However, the immunogenicity of compounds of mol wt of approximately 5000 or below has been reported (19–24). Two such compounds, α,*N*-DNP-hepta-L-Lysine, and a *p*-azo-benzenearsonate conjugate of hexa-L-tyrosine, which were found to be immunogenic (22, 23), have approximately the same molecular weight as that of the decapeptide which we found was not immunogenic although it does combine with anti-TMVP. It thus appears that the molecular weight necessary for immunogenicity may vary from system to system and is probably governed by the chemical nature of the determinant and/or the carrier.

The data presented herein show that the TMVP peptides and their derivatives elicited specific delayed and immediate skin reactions in guinea pigs immunized with TMVP. It should be noted that the concentration of TMVP peptides necessary to obtain a response was high when compared to the concentration of the whole antigen, TMVP, required. However, the responses were specific and occurred only in TMVP-sensitized animals. It is not clear

why only high challenging dosages of peptides elicited responses. Perhaps it is because binding of the peptides with humoral antibodies or with cellular receptors is low. Perhaps it is because at high concentrations the peptides aggregate, thus forming multivalent antigens. Also, high concentrations may enhance the adsorption of the peptides onto body proteins, thus forming either multivalent antigens or univalent antigens of large molecular weight with increased binding. It need not be assumed, however, that the high concentrations of peptides required indicates that aggregation with formation of multivalent antigens must occur for a skin reaction to appear. The necessity for using high concentrations of the peptides in our studies may also be due to the more rapid removal of small peptides from the injection site than when high molecular weight antigens are used.

The delayed hypersensitivity skin reactions which were elicited by the peptides were independent of carrier specificity. Delayed reactions were elicited with all of the peptides tested, but the lack of carrier specificity is most apparent in the ability of the *N*-acyl derivatives of the tripeptide to elicit reactions. The tripeptide Ala-Thr-Arg may, in this case, be considered the antigenic determinant or hapten. It may, however, be argued that even in this small peptide, one or two of the C-terminal amino acids may still constitute a portion of the carrier. Although this can not yet be excluded, it is rather unlikely since it has been shown (15) that octanoyl-Thr-Arg does not bind with antibodies to TMVP. It is generally accepted that the elicitation of delayed skin hypersensitivity reactions requires carrier specificity in contrast to immediate reactions which can be elicited equally well with many hapten-carrier conjugates (25–30). However, in some of the studies cited, delayed reactions could be elicited by the immunizing hapten conjugated to a carrier different from that used in the primary immunization if high antigen concentrations were used. This observation correlates with our findings that elicitation of delayed skin reactions, independent of carrier specificity, required high antigen dosages. An apparent absence of carrier specificity has been demonstrated by Leskowitz (24) using polytyrosine-*p*-azo benzenearsonate conjugates for immunization.

The results obtained in experiments dealing with the stimulation of ^{14}C-thymidine incorporation into spleen cells derived from TMVP-sensitized guinea pigs show that whereas TMVP was capable of stimulation, none of the peptides tested were stimulatory. It should be noted that the peptides were used in a wide range of concentrations, adjusted so that they approximately overlapped the concentrations of TMVP both on a weight basis and on a molar basis.

In some studies lymphocyte stimulation was produced by the immunizing hapten-protein conjugates, while the hapten conjugated to an unrelated carrier was ineffective (31, 32), indicating that carrier specificity plays a role in in vitro lymphocyte stimulation as well as in the in vivo response. Therefore, the lack of carrier specificity may account for the failure of the TMVP peptides to cause lymphocyte stimulation. If indeed in the TMVP system the carrier is essential

for stimulation, then it must be postulated that even the eicosapeptide either contains only part of the carrier (not sufficient to participate in stimulation) or that the carrier consists of other portions of the protein. However, others have shown that lymphocyte stimulation can be produced by a hapten conjugated to a protein different from that used in immunization (30).

The inability of the TMVP peptides to stimulate may be due to the possibility that even the relatively high concentrations of peptides which were in some of the cultures were not high enough to induce stimulation. Perhaps the binding of the peptides to receptor sites on the cells is so low that even these concentrations were insufficient. However, when the concentrations were increased 10–100-fold, the stimulation was not increased but rather decreased, probably due to cytotoxicity. A further possible explanation for the inability of the peptides to stimulate lymphocyte proliferation is that a multivalent antigen may be required.

Stulborg and Schlossman reported that only immunogenic members of a homologous series of α,DNP-L-lysines were capable of stimulation of sensitized lymphocytes (33). The results obtained with the TMVP peptides are in accord with these findings in that the TMVP peptides are nonimmunogenic, neither are they capable of stimulating TMVP-primed lymphocytes.

Although the peptides were not able to stimulate primed lymphocytes, they were able to elicit delayed skin reactions in TMVP-sensitized guinea pigs. These results differ from those described by several authors in which a direct correlation between the ability to stimulate primed lymphocytes and the ability to elicit delayed skin reactions has been noted (22, 33, 34).

Results reported in this communication further show that the TMVP peptides specifically inhibit the capillary migration of peritoneal exudate cells derived from TMVP-sensitized guinea pigs. These findings are in agreement with reports by others noting the correlation between delayed reactions and inhibition of capillary migration (18, 35, 36). In contrast to findings previously reported (37), the results reported herein demonstrate that carrier specificity is not essential for the inhibition of capillary migration. In fact, our finding that carrier specificity is not essential for inhibition of migration is in complete agreement with our demonstration of the lack of carrier specificity for the elicitation of delayed skin reactions.

The present results show that the peptides were capable of inhibiting capillary migration of sensitized peritoneal exudate cells, although they were not immunogenic. These findings contrast with those previously reported which correlated the ability to inhibit with immunogenicity (35). The results further show that although the peptides could inhibit capillary migration, they were unable to stimulate TMVP-primed lymphocytes. A similar lack of correlation has recently been reported: a carbohydrate fraction obtained from BCG cul-

ture filtrate elicited delayed skin reactions in BCG-sensitized animals (38), inhibited the capillary migration of peritoneal exudate cells derived from these animals (36), but failed to stimulate BCG-primed lymphocytes.[3] These observations differ from those reported by others who showed that the ability to inhibit capillary migration correlated with the ability to stimulate sensitized lymphocytes (33, 35).

In some respects our findings on the various parameters of the immune response tested with the TMVP system agree with those reported by others utilizing different systems, and in some respects they do not. The disagreements may well stem from the fact that the antigenic systems vary. However, the use of many well characterized antigenic systems is required in order to elucidate the common underlying principles governing the immune response.

SUMMARY

The following peptides have previously been shown to bind specifically with antibodies to TMVP: (*a*) An eicosapeptide representing residues 93–112 of TMVP and having the sequence Ileu-Ileu-Glu-Val-Glu-AspNH_2-GluNH_2-Ala-AspNH_2-Pro-Thr-Thr-Ala-Glu-Thr-Leu-Asp-Ala-Thr-Arg. (*b*) Its C-terminal decapeptide. (*c*) Its C-terminal pentapeptide. (*d*) *N*-octanoyl-C-terminal-tripeptide. (*e*) $(Lys)_4$-C-terminal-pentapeptide. (*f*) $(Lys)_7$ C-terminal-pentapeptide. The present communication deals with the investigation of several parameters of the immunological activity of the peptides. The results show that none of the peptides tested were immunogenic in guinea pigs, nor did they stimulate the incorporation of ^{14}C-thymidine by spleen cells derived from TMVP-primed animals. Results also showed that all of the peptides tested could elicit specific delayed and immediate skin reactions in TMVP-sensitized guinea pigs, and furthermore, that the peptides could specifically inhibit the migration of peritoneal exudate cells derived from these animals. The elicitation of delayed skin reactions and the ability to inhibit migration of peritoneal exudate cells were independent of carrier specificity.

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