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Synthetic Antigens Composed Exclusively of L- or D-Amino acids

I. EFFECT OF OPTICAL CONFIGURATION ON THE IMMUNOGENICITY OF SYNTHETIC POLYPEPTIDES IN MICE

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Summary. Two random linear co-polymers composed exclusively of D-amino acids were found to elicit a primary response in mice; the sera were assayed by the indirect precipitin reaction. 247, *p* (D-Tyr, D-Glu, D-Ala) was studied intensively and compared to a similar polypeptide, 253, *p* (L-Tyr, L-Glu, L-Ala), of opposite optical configuration. The primary response to the D-isomer in adjuvant is highly dose-dependent, showing a sharp maximum around 1 μg/mouse, while that to the L-isomer in adjuvant is largely dose-independent. At low doses the D-isomer is as immunogenic as the L-isomer. Re-injection of the D-isomer led to no increase in titre; re-injection of the L-isomer gave a typical secondary response. The antibodies formed to both isomers were highly stereospecific. The D-isomer was 50–1000 times as efficient at inducing paralysis as the L-isomer, and the paralysis was specific. The differences between the D- and L-isomers are thought to be explained by the much greater capacity of the D-isomer in inducing immunological paralysis.

INTRODUCTION

The development of synthetic polypeptide antigens in recent years (for review see Sela, 1966) has led to extensive studies of factors controlling immunogenicity in protein antigens. The synthetic polypeptides serve as model systems whose variations are more readily controlled than those of naturally occurring proteins. One feature that may be varied is the optical configuration of the component amino acids.

It has been shown repeatedly that antibodies are capable of distinguishing optical isomers of amino acids contained in antigenic determinants (Sela, 1966). It is also known that attachment of D-tyrosine to gelatin, or incorporation of D-tyrosine into random copolymers or branched polymers of L-amino acids leads to a more immunogenic material, or may convert a non-immunogen into an immunogen; the antibodies elicited are largely sterospecific (Sela, 1966). However, attempts to demonstrate immune responses to synthetic polypeptides composed exclusively of D-amino acids in rabbits and guinea-pigs (Gill, Gould and Doty, 1963; Maurer, 1963; Sela and Fuchs, 1965; Borek, Stupp, Fuchs and Sela, 1965; Maurer, 1965; Ben-Efraim, Fuchs and Sela, 1967), and in mice and men (Maurer, 1965) have repeatedly met with failure, while the corresponding polypeptides of L-amino acids were immunogenic. Only one report has been published that a synthetic polypeptide composed of D-tyrosine, D-glutamic acid and D-lysine was immunogenic in

rabbits (Gill, Gould, Kunz and Doty, 1964) by the precipitin reaction, although it was less immunogenic than the corresponding polypeptide composed of L-amino acids.

It was of interest to study the differences in immunological behaviour of polypeptides composed entirely of D- or L-amino acids, but which were otherwise comparable. A detailed knowledge of these differences might lead to an increased understanding of the process by which antigen stimulates cells to synthesize antibody. Therefore, we tested two polypeptides composed exclusively of D-amino acids for immunogenicity in mice, and compared the response to one of them with that to a similar polypeptide composed exclusively of L-amino acids.

MATERIALS AND METHODS

Animals

CBA × C57 BL F_1 mice of both sexes aged 6–12 months, bred from the parental strains maintained at the National Institute for Medical Research, London, were used in all the experiments.

TABLE 1

PROPERTIES OF THE SYNTHETIC POLYPEPTIDES AND PEPTIDYL GELATINS USED IN THESE EXPERIMENTS

No. and designation of the sample	Text abbreviation	Molar proportions of amino acid residues in the polypeptide	Average molecular weight	Per cent tyrosine by weight
247, *p* (D-Tyr, D-Glu, D-Ala)	D-TGA 247	1:5·6:4·35	19,700	13·7
253, *p* (L-Tyr, L-Glu, L-Ala)	L-TGA 253	1:6·4:5·7	23,000	11·7
251, *p* (D-Tyr, D-Glu, D-Lys)	D-TGL 251	1:10·4:6	44,000	7·3
1011, *p* (D-Tyr, D-Glu)		1:2·5	27,000	33·7
595a, *p* (D-Tyr, D-Glu)-Gelatin*				7·6†
246 *p*-D-Tyr-Gelatin				13·2†
240, *p*-L-Tyr-Gelatin				13·5†

* This preparation was enriched with 7·6 per cent of tyrosine residues and 14·7 per cent of glutamic acid residues, assuming the unmodified gelatin to be 100 per cent.

† These values give the enrichment of gelatin with tyrosine residues, and do not take into consideration any small amount of tyrosine in the original gelatin; the unmodified gelatin is assumed to be 100 per cent.

Antigens

The synthesis and characterization of the polypeptides listed in Table 1 were carried out according to the methods described by Sela, Fuchs and Arnon (1962). The physical and chemical properties of the polymers composed exclusively of D-amino acids which were used as immunogens have been described, with their immunological activities in guinea-pigs and rabbits, by Sela and Fuchs (1965) and Borek *et al.* (1965). The peptidyl gelatins were prepared according to Arnon and Sela (1960). In the text we shall use the simplified abbreviations given in Table 1. Concentrations of immunizing and ^{125}I-labelled test antigen solutions were determined spectrophotometrically.

Immunization

All immunization was carried out via the hind footpads. Low dilutions of antigen were prepared in a 0·5 per cent solution of a low molecular weight gelatin (batch No. 180, the

British Gelatin and Glue Research Association) in phosphate buffered saline (0·145 M NaCl, 0·014 M potassium phosphate buffer, pH 7·4). Antigen was administered either in gelatin–saline solution, adsorbed on alum and mixed with 10^8 killed *Bordetella pertussis* organisms, which act as an adjuvant, or emulsified in complete Freund's adjuvant (1 part antigen in gelatin–saline solution; 1 part lanolin; 2 parts liquid paraffin; with a final concentration of 2 mg/ml *Mycobacterium tuberculosis*). The mice were bled from the retro-orbital plexus, at various times as stated in the text, and the serum was separated from the clot and freed from red cells by centrifugation. Sera were either titred immediately, or stored in tightly capped tubes at −20° until used. In the experiments in which it was attempted to induce paralysis, the mice were injected with various doses of antigen in gelatin–saline solution as above, bled individually at 14 days, and given a challenge injection of antigen in complete Freund's adjuvant 1 or 2 days later.

Antibody assay

All sera were titred individually in terms of their antigen binding capacity. Five microlitres of each serum was incubated with a given amount of antigen, trace labelled with ^{125}I by the method of Greenwood, Hunter and Glover (1963), in about 0·7 ml 1 per cent bovine serum albumin solution in phosphate buffered saline (0·145 M NaCl, 0·014 M potassium phosphate buffer, pH 7·4) for 1 hour at 37°. It was found empirically that in this system the presence of bovine serum albumin helped to lower the control values. Then a slight excess of rabbit anti-mouse–immunoglobulin G antiserum was added in an amount sufficient to precipitate all the mouse antibody present, and to bring the volume to 0·75 ml. The tubes were incubated for a further hour at 37°, and then all the tubes were counted in a well-type scintillation counter. After overnight incubation at 4° the tubes were centrifuged, the supernatant fluid decanted, the tubes drained and swabbed to remove unbound antigen, and the undissolved precipitates counted. Control serum was obtained from a group of mice injected with 0·5 per cent gelatin in saline solution emulsified in complete Freund's adjuvant and bled out at 3 weeks. Provided freshly prepared albumin solution was used, antigen binding in controls was about 1 per cent. The rabbit anti-mouse immunoglobulin G antiserum used was prepared by immunizing rabbits with a partially purified 7S γ_2-mouse myeloma protein (MP 5563) as described by McDevitt and Sela (1965).

The titres are expressed as per cent of a given quantity of antigen bound per millilitre serum, after subtracting the control value. By varying the amount of antigen added to a given amount of a single serum, a curve like that shown in Fig. 1 may be obtained (similar curves are generated by using a fixed amount of antigen and diluting the test serum in normal mouse serum); curves like this can be used to obtain a rough estimate of the relative antibody in each of a set of sera all titred at a single antigen concentration. Titration of each of a set of sera at several concentrations of antigen show that this method of estimation gives quite consistent results.

To study the cross-reactions of sera, two techniques were used. In the first, the antigen binding capacity of a serum was assayed with heterologous radio-labelled antigen as with homologous antigen. In the second, sera were added to mixtures of a given amount of radio-labelled antigen and varying amounts of the unlabelled antigen being tested for cross-reaction. The ratio was varied from 1:1 to 1:1000 and the per cent of the labelled antigen bound determined as above. This second technique was also used to verify that the trace labelled and unlabelled antigen reacted identically with antiserum.

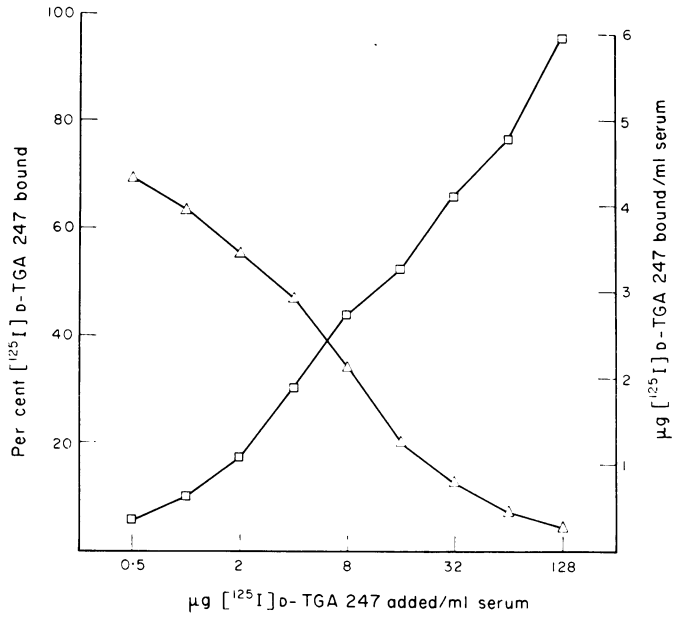

FIG. 1. Binding of [^{125}I]D-TGA 247. Varying amounts of [^{125}I]D-TGA 247 were added to 10 μl of a pool of serum from mice bled 21 days after a primary injection of 1 μg D-TGA 247 in complete Freund's adjuvant. △, Per cent [^{125}I]D-TGA 247 bound; □, μg [^{125}I]D-TGA 247 bound/ml serum, obtained as the product of per cent [^{125}I]D-TGA 247 bound and μg [^{125}I]D-TGA 247 added/ml serum.

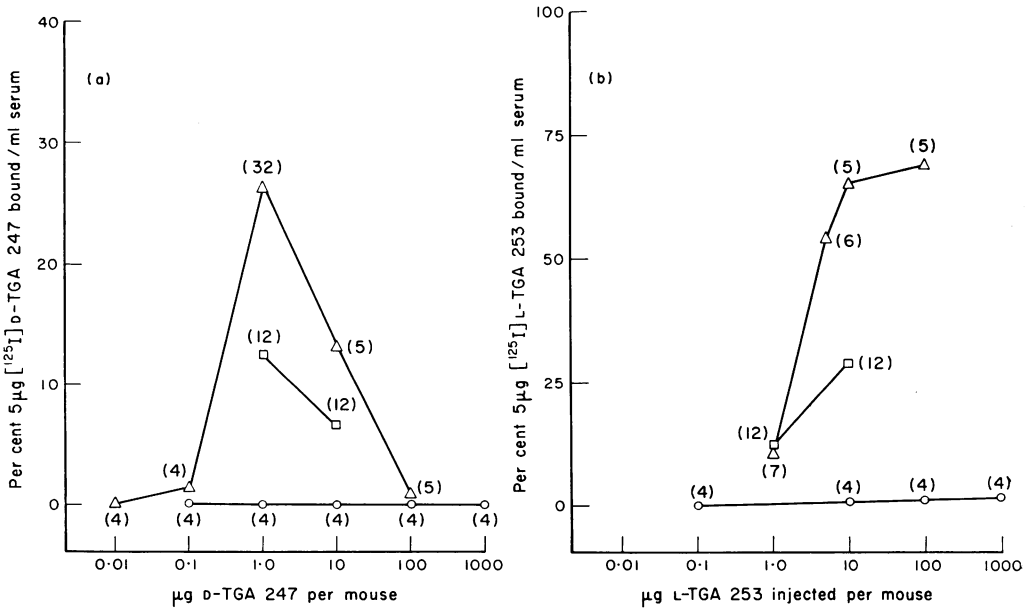

FIG. 2. The effect of dose on the primary response to D-TGA 247 and L-TGA 253. The numbers in parentheses give the number of animals whose titres were averaged at each point. (a) Primary response to D-TGA 247: ○, in saline, 14 days; △, in complete Freund's adjuvant, 25 days; □, alum precipitated +pertussis, 21 days. (b) Primary response to L-TGA 253: ○, in saline, 14 days; △, in complete Freund's adjuvant, 21 days; □, alum precipitated +pertussis, 21 days.

RESULTS

PRIMARY RESPONSE

D-TGA 247 and D-TGL 251 both elicit a definite primary response in mice when injected in complete Freund's adjuvant. The effect of dose on the primary response to D-TGA 247 is illustrated in Fig. 2(a). All mice in two groups immunized with 1 or 10 μg D-TGL 251 in complete Freund's adjuvant responded, binding 10–20 per cent of 5 μg[^{125}I]D-TGL 251/ml serum. It is apparent that the primary response to D-TGA 247 in adjuvant is highly dose-dependent, with a maximum around 1 μg D-TGA 247 per mouse. On the other hand, the primary response to L-TGA 253 (Fig. 2b), the comparable polypeptide

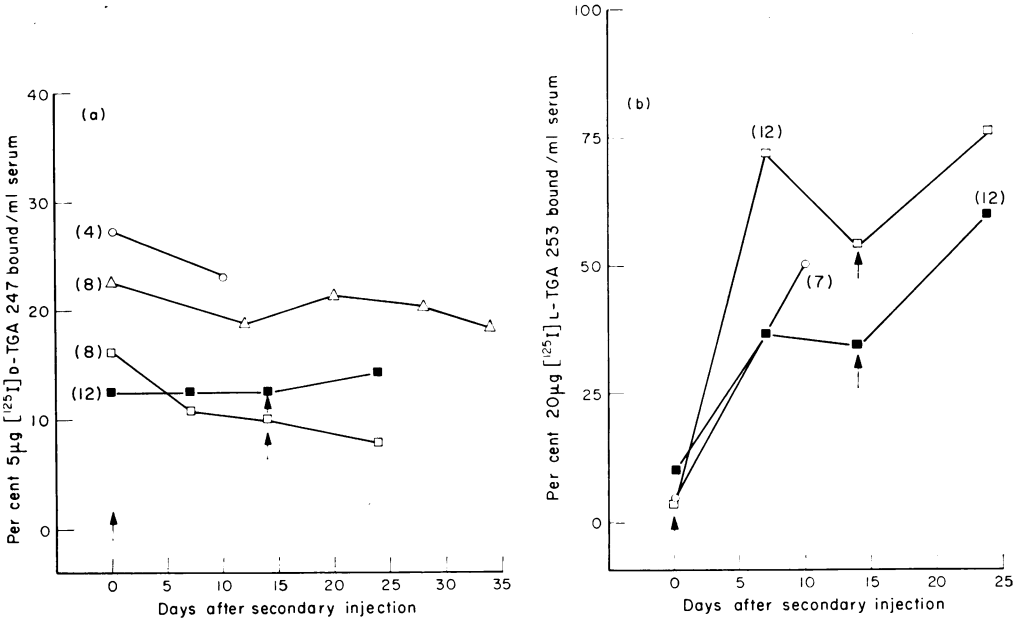

FIG. 3. Effect of secondary injections on the response to D-TGA 247 and L-TGA 253. The vertical arrow marks the time of injection. Numbers in parentheses give the number of animals whose titres were averaged for each experiment. Titres at 0 days are titres just prior to secondary injection. (a) Secondary injections of D-TGA 247. Mice primed with 1 μg D-TGA 247 in complete Freund's adjuvant and boosted at 27 days with 1 μg D-TGA 247: ○, in saline; △, in complete Freund's adjuvant; □, alum precipitated + pertussis, repeated at 14 days; ■, mice primed with 1 μg alum precipitated TGA 247 + pertussis, and boosted with the same after 27 and 41 days. (b) Secondary injections of L-TGA 253. Mice primed with alum precipitated L-TGA 253 + pertussis, and boosted with the same after 27 and 41 days: □, 1 μg; ■, 10 μg; ○, Mice primed with 1 μg L-TGA 253 in complete Freund's adjuvant and boosted with 100 μg L-TGA 253 in saline after 26 days.

composed exclusively of L-amino acids, in complete Freund's adjuvant, is relatively dose-independent above 5 μg/mouse. It is noteworthy that at a dose of 1 μg/mouse in complete Freund's adjuvant there is little difference between the primary response to D-TGA 247 and that to L-TGA 253. There was no primary response to D-TGA 247 in saline and only a minimal response to L-TGA 253 in saline.

SECONDARY RESPONSE

Fig. 3(a) shows the effect of re-injection on the response to D-TGA 247. None of these attempts led to any increase in antibody titre. In one experiment, where mice were primed with 1 μg D-TGA 247 in complete Freund's adjuvant and boosted with the same after 24 days, one mouse out of nine showed a 2·5-fold increase in per cent antigen bound.

On the other hand, Fig. 3(b) shows the effect of re-injection on the response to L-TGA 253. Repeated injections of L-TGA 253 in complete Freund's adjuvant were not employed since a secondary response is readily elicited with less powerful adjuvants. The effect of primary injection of antigen in saline on the response to subsequent injections will be referred to later.

SPECIFICITY OF ANTIBODIES

Several individual sera and serum pools from mice giving a primary response to D-TGA 247 were assayed at several concentrations of [^{125}I]D-TGA 247, and it was found that at the lowest concentrations up to 85 per cent of the [^{125}I]D-TGA 247 was specifically bound by these antisera; therefore antibody is present against determinants found on the majority, and perhaps all, of the molecules of D-TGA 247. No antiserum from mice immunized with D-TGA 247 bound any [^{125}I]L-TGA 253. Of all the compounds listed in Table 1, only

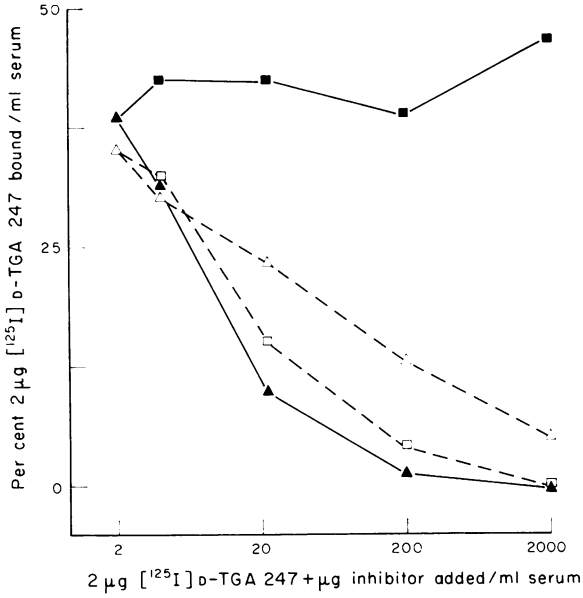

FIG. 4. Cross-reactions between D-TGA 247 and L-TGA 253. Binding of [^{125}I]D-TGA 247 by anti-D-TGA 247 antiserum in the presence of varying amounts of ▲, D-TGA 247; ■, L-TGA 253. Binding of [^{125}I]D-TGA 247 by anti-L-TGA 253 antiserum in the presence of varying amounts of: △, D-TGA 247; □, L-TGA 253.

D-TGA 247 inhibited binding of [^{125}I]D-TGA 247 by these antisera, even at a 1000:1 ratio of inhibitor to [^{125}I]D-TGA 247. The fact that a mixture of [^{125}I]D-TGA 247 and D-TGA 247 is bound to the same extent by these antisera as an equal amount of [^{125}I]D-TGA 247 implies that labelling of the test antigen does not alter its binding by antibody.

On the other hand, an occasional high-titre anti-L-TGA 253 antiserum would bind a small amount of [^{125}I]D-TGA 247; the capacity of these sera to bind [^{125}I]D-TGA 247 was less than 1 per cent of their capacity to bind [^{125}I]D-TGA 253. Nevertheless, at the lowest concentrations of antigen up to 50 per cent of the [^{125}I]D-TGA 247 could be bound by these antisera; therefore they were binding at least some of the molecules bound by anti-D-TGA 247 antisera. However, binding of [^{125}I]D-TGA 247 by anti-L-TGA 253 is inhibited more strongly by L-TGA 253 than by D-TGA 247, as shown in Fig. 4.

Binding of [^{125}I]L-TGA 253 by anti-L-TGA 253 is not measurably inhibited by D-TGA 247 even at 1000 times the amount of [^{125}I]L-TGA 253. Therefore, only a very small proportion of the anti-L-TGA 253 antibodies appear to be capable of binding D-TGA 247, or else the cross-reaction with D-TGA 247 involves binding of very low affinity.

INDICATION OF PARALYSIS

Paralysis to D-TGA 247 and L-TGA 253 was induced in adult mice as described in 'Methods'. Doses of D-TGA 247 in saline ranged from 0·1 μg to 1·0 mg. At 17 days the mice were challenged with 1 μg D-TGA 247 in complete Freund's adjuvant to elicit a maximal response, and they were bled 21 days later. Results are shown in Fig. 5(a).

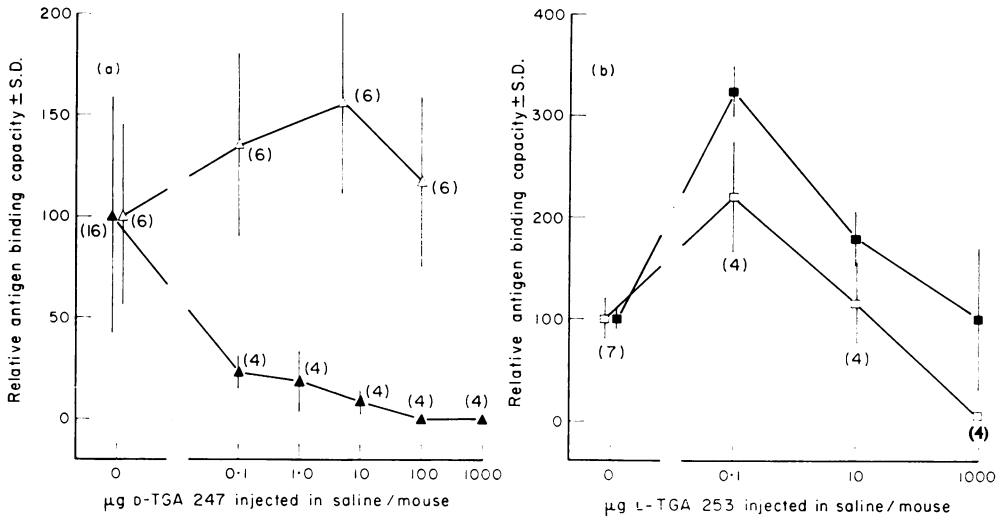

FIG. 5. Induction of paralysis with D-TGA 247 and L-TGA 253. Average titres are expressed as per cent of the untreated control (100 per cent) ± standard deviation, and they were calculated as described in 'Methods'. Numbers in parentheses are number of animals per group. (a) D-TGA 247 injected in saline 17 days before challenge with: ▲, 1 μg D-TGA 247 in complete Freund's adjuvant, 21-day titres; △, 5 μg L-TGA 253 in complete Freund's adjuvant, 21-day titres. (b) L-TGA 253 injected in saline 14 days before challenge with: ■, 1 μg L-TGA 253 in complete Freund's adjuvant, 20-day titres; □, 36-day titres, 10 days after boosting with 100 μg L-TGA 253 in saline.

One hundred micrograms of D-TGA 247 paralyse completely to subsequent challenge, and even 0·1 μg gives a 75 per cent suppression of the response. A similar experiment (Fig. 5a), in which challenge was with 5 μg L-TGA 253 in complete Freund's adjuvant showed the paralysis to be specific for D-TGA 247; challenge of these groups of mice with D-TGA 247 gave the same result as before. Doses of L-TGA 253 in saline ranged from 0·1 μg to 1 mg. At 14 days the mice were challenged with 1 μg L-TGA 253 in complete Freund's adjuvant. The responses at 20 days (Fig. 5b) were rather low, so the mice were boosted with 100 μg L-TGA 253 in saline. This increased the titres of the untreated group 25-fold at 10 days, but not those of the group pre-treated with 1 mg TGA 253. This was taken to indicate that a partial unresponsiveness had been induced in the animals in this group.

A direct comparison of the efficiency in inducing paralysis of D-TGA 247 and L-TGA 253 is quite difficult, because L-TGA 253 in saline at low doses actually enhances the response to subsequent challenge. However, one can say, on the basis of the partial

inhibition of the response to challenge with L-TGA 253 induced by pre-treatment of the mice with 1 mg L-TGA 253 in saline, that D-TGA 247 is at very least fifty times more efficient than L-TGA 253 in inducing paralysis.

DISCUSSION

We have shown that polypeptides composed exclusively of D-amino acids can elicit an antibody response in mice. The antibodies are highly specific for the D-configuration. The response is not due to contamination of the preparation with entire molecules in the L-configuration. This is apparent from the data on dose-response, the lack of cross-reactions and the ability of these antisera to bind almost all the [^{125}I]D-TGA 247. Furthermore, it has been shown in rabbits that when polymers composed exclusively of D-amino acids are intentionally 'contaminated' with as little as 3 per cent L-amino acids, they become immunogenic in rabbits, in contrast to the exclusively D-amino acid polymers (Y. Stupp and M. Sela, unpublished data). Both the polypeptides composed exclusively of D-amino acids that were used in these experiments have been found not to be immunogenic in the same system (Sela and Fuchs, 1965; Borek *et al.*, 1965), so they presumably contain less than 3 per cent amino acids in the L-configuration. The ability of anti-L-TGA 253 antisera to bind [^{125}I]D-TGA 247 is not entirely unexpected; Maurer (1963) has observed a similar cross-reaction.

Gill *et al.* (1964) (confirmed recently by Y. Stupp and M. Sela, unpublished data), found that a compound very similar to D-TGL 251 was immunogenic in rabbits. We are now able to confirm this result for D-TGL 251 in mice. This leads to a consideration of why other polypeptides composed exclusively of D-amino acids were found not to be immunogenic (Gill *et al.*, 1963; Maurer, 1963; Sela and Fuchs, 1965; Borek *et al.*, 1965; Maurer, 1965; Ben-Efraim *et al.*, 1967). There are several possible explanations: the polypeptides may actually not be immunogenic; the animals may be genetically unable to respond; the immunizing dose and schedule may not have been optimal; or the means of testing may not have been able to detect the response. From our results we are unable to say anything about the first two possibilities, except that mice appear to be able to respond to some polypeptides composed exclusively of D-amino acids. Gill *et al.* (1964) and Stupp and Sela (unpublished data) have shown that rabbits also can respond to other polypeptides composed exclusively of D-amino acids. In mice weighing roughly 30 g, the optimal dose was about 1 μg in complete Freund's adjuvant, or 33 μg/kg. Furthermore, subsequent injections failed to increase and sometimes decreased the antibody levels. Therefore polypeptides composed exclusively of D-amino acids appear to be best tested for immunogenicity over a wide dose range, including quite low doses, and antibodies looked for in the primary response. The method used for detecting antibody is also quite sensitive, being able to detect 1 μg antibody/ml serum quite readily; it has the further advantage of depending on a primary reaction of antibody with antigen—unlike precipitation and passive cutaneous anaphylaxis, which involve more than simple binding of antigen. It is possible that the antibodies we detected in sera by antigen binding are not capable of precipitation or of production of passive cutaneous anaphylaxis.

Several explanations have been advanced to account for the apparent non-immunogenicity of polypeptides composed exclusively of D-amino acids (Sela, 1966). In these experiments the very fact of the immunogenicity of D-TGL 251 and D-TGA 247 make several of them inapplicable. It is evident that antibody sites can be synthesized which can

interact with determinants in the D-configuration, and that the animals are genetically equipped to produce such antibody, although this may not be true for other species and other polypeptides; only positive results can resolve this. Furthermore, because these polypeptides are immunogenic, we assume that they are able to reach the crucial site in the induction of the specific antibody response. Gill, Papermaster and Mowbray (1964, 1965) and Janeway and Humphrey (unpublished) have shown that polypeptides composed exclusively of D-amino acids can be slowly degraded *in vivo*. Zubay (1964) proposed that the reason for the apparent lack of immunogenicity of polypeptides composed exclusively of D-amino acids was a high persisting level of relatively undegradable antigen, leading to immunological paralysis, and advocated testing the polypeptides at very low doses. We believe that a sufficient explanation of the differences we observed between the immunological behaviour of L-TGA 253 and that of D-TGA 247 is the high capacity of D-TGA 247 to induce immunological paralysis. The fact that as little as 0·1 μg D-TGA 247 in saline, a dose producing a barely detectable primary response in adjuvant, can lead to a 75 per cent suppression of the response to subsequent challenge is quite remarkable. The fact that D-TGA 247 becomes less immunogenic with increasing dose above 1 μg, and that no secondary response, nor even a second primary type response, can be observed, can be readily explained by the hypothesis that any encounter of an animal with D-TGA 247 leads to paralysis of a proportion of the immunologically competent cells, and stimulation of the remainder. This in turn appears likely to be due to the high persistence in tissue and low rate of metabolic breakdown of D-TGA 247 (Janeway and Humphrey, 1967). It also seems that there is no inherent difference in immunogenicity between D-TGA 247 and L-TGA 253. The observed differences in immunogenicity above a dose of 1 μg appear to be due to the supervening induction of paralysis by D-TGA 247.

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REFERENCES

ARNON, R. and SELA, M. (1960). 'Studies on the chemical basis of the antigenicity of proteins. II. Antigenic specificity of polytyrosyl gelatins.' *Biochem. J.*, **75**, 103.

BEN-EFRAIM, S., FUCHS, S. and SELA, M. (1967). 'Differences in immune response to synthetic antigens in two inbred strains of guinea-pigs.' *Immunology*, **12**, 573.

BOREK, F., STUPP, Y., FUCHS, S. and SELA, M. (1965). 'Relations between optical configuration and immunogenicity of synthetic polypeptides.' *Biochem. J.*, **96**, 775.

GILL, T. J. III, GOULD, H. J. and DOTY, P. (1963). 'Role of optical isomers in determining the antigenicity of synthetic polypeptides.' *Nature (Lond.)*, **197**, 746.

GILL, T. J. III, KUNZ, H. W., GOULD, H. J. and DOTY, P. (1946). 'Studies on synthetic polypeptide antigens. XI. The antigenicity of optically isomeric synthetic polypeptides.' *J. biol. Chem.*, **239**, 1107.

GILL, T. J. III, PAPERMASTER, D. S. and MOBRAY, J. F. (1964). 'Metabolism of isomeric synthetic polypeptides.' *Nature (Lond.)*, **203**, 644.

GILL, T. J. III, PAPERMASTER, D. S. and MOWBRAY, J. F. (1964). 'Synthetic polypeptide metabolism. I. The metabolic fate of enantiomorphic polymers.' *J. Immunol.*, **95**, 794.

GREENWOOD, F. C., HUNTER, W. M. and GLOVER, J. S. (1963). 'The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity.' *Biochem. J.*, **89**, 114.

MAURER, P. H. (1963). 'Antigenicity of polypeptides (poly alpha amino acids). X. Studies with polymers of D-amino acids.' *Proc. Soc. exp. Biol. (N.Y.)*, **113**, 553.

MAURER, P. H. (1965). 'Antigenicity of polypeptides (poly alpha amino acids). XIII. Immunological studies with synthetic polymers containing only D- or D- and L- alpha amino acids.' *J. exp. Med.*, **121**, 339.

MCDEVITT, H. O. and SELA, M. (1965). 'Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains of inbred mice.' *J. exp. Med.*, **122**, 517.

SELA, M. (1966). 'Immunological studies with synthetic polypeptides.' *Advanc. Immunol.*, **5**, 29.

SELA, M., FUCHS, S. and ARNON, R. (1962). 'Studies on the chemical basis of the antigenicity of proteins. V. Synthesis, characterization, and immunogenicity of some multichain and linear polypeptides containing tyrosine.' *Biochem. J.*, **85**, 223.

SELA, M. and FUCHS, S. (1965). 'On the role of charge and optical configuration in antigenicity.' *Symposium on the Molecular and Cellular Basis of Antibody Formation* (Ed. by J. Sterzl), p. 43. Publishing House of Czechoslovak Academy of Sciences, Prague.

ZUBAY, G. (1964). 'Apparent inability of polypeptides constructed from D-amino acids to stimulate antibody formation.' *Nature (Lond.)*, **200**, 483.