STUDIES ON SYNTHETIC POLYPEPTIDE ANTIGENS

IV. THE METABOLIC FATE OF THE ANTIGEN

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SUMMARY

The metabolic fate of the synthetic polypeptide antigen $G_{56}L_{38}T_6$ following intravenous injection in the rabbit has been studied. The response to the primary injection of the antigen was either the usual three-phase elimination pattern associated with the virgin exposure to a heterologous protein antigen or the rapid elimination pattern associated with previous exposure to the antigen. On subsequent injections of the antigen, however, all of the animals showed the usual type of accelerated antigen disappearance.

The related synthetic polypeptides $G_{59}L_{41}$ and G_{100} elicited no anamnestic response in animals previously sensitized to $G_{56}L_{38}T_6$.

INTRODUCTION

Previous studies in this series[1-3] have shown that a group of linear-chain synthetic polypeptides containing different combinations of L-glutamic acid, L-lysine, L-tyrosine and L-phenylalanine can elicit the production of precipitating antibodies in the rabbit. The present study was undertaken to investigate the metabolic fate of the most potent of these antigens, $G_{56}L_{38}T_6$ (56.4% glutamic acid, 37.8% lysine and 5.8% tyrosine), and to study the ability of the comparatively weak antigen $G_{59}L_{41}$ (59.0% glutamic acid and 41.0% lysine) and the non-antigenic G_{100} (polyglutamic acid) to elicit an anamnestic response in rabbits previously sensitized with $G_{56}L_{38}T_6$. The influence of prior sensitization with an antigen on enhancing the circulating antibody response to a second antigen has been demonstrated in the rabbit, man and the ferret[6-9].

METHODS

The metabolic fate of $G_{56}L_{38}T_6$ was followed by use of the radio-iodinated antigen. Using the method of TALMAGE *et al*[10]. the efficiency of iodination of the synthetic polypeptide was 48% and an average of 1.8 atoms of iodine was added to each molecule of $G_{56}L_{38}T_6$.

Nine albino rabbits weighing 2–2.5 kg each were injected intravenously with 60 mg

of radio-iodinated $G_{56}L_{38}T_6$ after a preparatory period of 4 days during which they received 0.5% KI in their drinking water to saturate the iodine-utilizing tissues. The animals were bled daily from the marginal ear vein, heparin was added to the blood, and the plasma was preserved with merthiolate (1:10000). When the antigen remaining in the plasma had fallen below 0.1% of the administered antigen, a second injection of 10 mg of radio-iodinated $G_{56}L_{38}T_6$ was given intravenously to determine its fate in the presence of circulating antibody (sensitized response). Following a period of 7–8 weeks, when no precipitating antibody could be detected in the plasma, 60 mg of radio-iodinated $G_{56}L_{38}T_6$ was given intravenously to determine its behavior in eliciting an anamnestic response.

The radioactivity was measured in a well-type scintillation counter and corrected for decay. The plasma volume was taken as 2.55% of the body weight[11]. The amount of antibody in the plasma was determined as previously described[3].

In order to study the ability of related synthetic polypeptides to elicit an anamnestic response, ten rabbits were initially injected intravenously with 60 mg of $G_{56}L_{38}T_6$, followed 15 days later by another 10 mg of $G_{56}L_{38}T_6$. Seven weeks after the second injection, when no precipitating antibodies were present in the plasma, five animals each received 60 mg of $G_{59}L_{41}$ or G_{100} intravenously. They were bled at intervals for 15 days and the plasma tested for the presence of precipitating antibodies with $G_{56}L_{38}T_6$, $G_{59}L_{41}$ and G_{100}.

RESULTS

The response to the primary injection of radio-iodinated $G_{56}L_{38}T_6$ fell into three groups, each of which consisted of three rabbits. The results are shown in Fig. 1. Group I showed the usual primary elimination pattern for a heterologous protein[11] with the antibody response beginning on the eighth day. Group II showed rapid elimination of the antigen within five days and the appearance of antibody on the fifth day. Group III showed gradual elimination of the antigen by twelve days but a marked antibody response on the fourth day. The antigen disappearance rate[11] for the 2–4 day period was 24% for group I, 72% for group II and 45% for group III. An antigen disappearance rate of less than 43% has been interpreted as signifying a virgin response to an antigen and a rate greater than 43%, a sensitized or anamnestic response[11]. Using starch gel electrophoresis[12], no difference in the plasma protein patterns was observed that could be correlated with the difference in rate of elimination of the antigen.

The sensitized and anamnestic responses were the same for all of the animals despite differences in their initial response. These are shown in Fig. 2. The antigen elimination rate for the 2–4 day period was 75% for the sensitized response and 90% for the anamnestic response.

$G_{56}L_{38}T_6$ was used to sensitize rabbits to be studied for the possibility that the related polypeptides $G_{59}L_{41}$ and G_{100} might elicit an anamnestic response. All of the animals showed antibody formation following the initial immunization with $G_{56}L_{38}T_6$, but neither $G_{59}L_{41}$ nor G_{100} was able to elicit an anamnestic response in these sensitized animals as judged by the absence of precipitating antibodies to $G_{56}L_{38}T_6$, $G_{59}L_{41}$, and G_{100}.

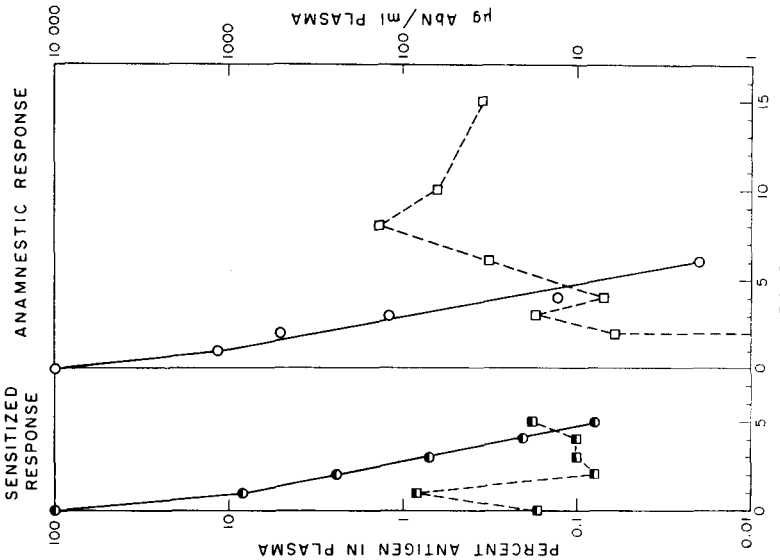

Fig. 2. The response of rabbits to $G_{56}L_{38}T_6$ in the presence of circulating antibodies (sensitized response) and after all the antibodies elicited by the first and second injections of the antigen had disappeared from the plasma (anamnestic response). The circles and solid lines represent the antigen elimination from the plasma, and the squares and broken line, the precipitating antibody in the plasma.

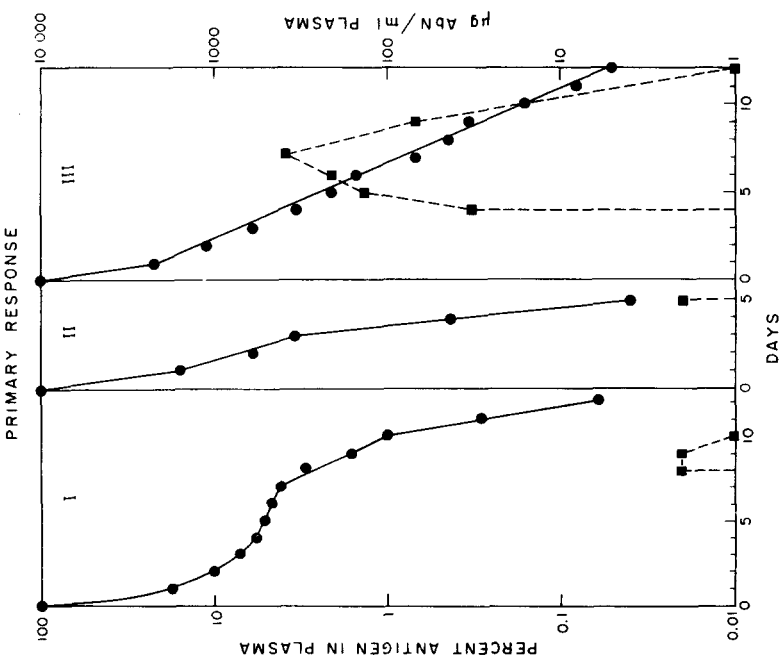

Fig. 1. The response of rabbits to the primary exposure to the synthetic polypeptide antigen $G_{56}L_{38}T_6$. Group I shows the usual three-phase elimination pattern associated with the virgin response to a native protein antigen, while groups II and III show the pattern of previously sensitized animals. The circles and solid lines represent the antigen elimination from the plasma, and the squares and broken line, the precipitating antibody in the plasma.

DISCUSSION

The primary response to intravenous immunization with $G_{56}L_{38}T_6$ was of two types: the three-phase elimination pattern typical of virgin stimulation with a native protein (group I) and the rapid elimination pattern seen in animals previously sensitized to the antigen (groups II and III). The simultaneous presence of antibody and antigen in the plasma when 5% of the radio-iodinated antigen was still present and the gradual elimination of the last few percent of the radioactivity from the plasma in group III are probably due to the presence of circulating antibody–antigen complexes. The precipitate is quite soluble at 37° (see ref. 3) and thus, in the case of a strong antibody response, the formation of circulating antibody–antigen complexes with a large excess of antibody would be expected. The presence of such complexes has been shown in the plasma during the primary response[13].

An explanation for the rapid elimination of $G_{56}L_{38}T_6$ in groups II and III on first exposure to the antigen might be sought in terms of the rapid primary elimination pattern seen with heavily modified proteins[14] or in terms of a sensitized response due to immunization with a related antigen prior to administration of the synthetic polypeptide. The lack of extensive conformational structure[15] renders $G_{56}L_{38}T_6$ comparable to a heavily modified or denatured protein; yet the fact that the animals in group I showed the usual type of elimination pattern following virgin exposure to a native protein antigen with antibody detectable on the eighth day belies the former explanation. On the other hand, groups II and III not only showed rapid elimination of the antigen, but also showed antibody detectable in the plasma on the fifth and fourth days respectively. Appearance of antibody at this time is earlier than usual for the primary response to a protein antigen, where antibody generally appears on the sixth day or later[11,14,16]. Thus, the reason for the variation in the primary response seems to lie within the animals rather than with the antigen. It is interesting to note in this respect that following the primary exposure to $G_{56}L_{38}T_6$, all the animals responded in the same fashion to subsequent injections of the antigen; the somewhat slower antigen disappearance rate calculated for the sensitized response than for the anamnestic response may have been due to antigen retention in the form of circulating antibody–antigen complexes in the former case. The possibility of previous stimulation by a related native protein antigen is made plausible by the simple chemical composition of the synthetic antigen. This simple composition affords an opportunity for the formation of many determinant sites containing different permutations and combinations of common amino acids, some of which sites may well be found in native proteins. Indeed, STAHMANN *et al.*[5,17] have shown that antisera to synthetic polypeptides cross-reacted with a wide range of polypeptidyl proteins and native proteins.

In both the sensitized and anamnestic responses, an initial increase in circulating antibody is followed by a fall and then a second rise. The initial rise may be due to the release of preformed antibody from the cells and the second rise, to the production of new antibody stimulated by the injection of antigen[9].

Under the same conditions in which rabbits immunized with $G_{56}L_{38}T_6$ showed a marked anamnestic response to $G_{56}L_{38}T_6$, none of them showed an anamnestic response to $G_{59}L_{41}$ or to G_{100}. In view of a possible "priming" effect of the previous immunization with $G_{56}L_{38}T_6$ (see refs. 6–9), however, it was felt that one or both might elicit antibodies that would react with one of these synthetic polypeptides.

The reason for the failure of either $G_{59}L_{41}$ or G_{100} to elicit an anamnestic response must await the further elucidation of the role of tyrosine in the induction of the antibody response.

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REFERENCES

1 T. J. GILL, III AND P. DOTY, *J. Mol. Biol.*, 2 (1960) 65.
2 T. J. GILL, III AND P. DOTY, *J. Clin. Invest.*, 40 (1961) 1042.
3 T. J. GILL, III AND P. DOTY, *J. Biol. Chem.*, 236 (1961) 2677.
4 M. A. STAHMANN, H. TSUYUKI, K. WEINKE, C. LAPRESLE AND P. GRABAR, *Compt. rend.*, 241 (1955) 1528.
5 D. J. BUCHANAN-DAVIDSON, M. A. STAHMANN, C. LAPRESLE AND P. GRABER, *J. Immunol.*, 83 (1959) 552.
6 F. J. DIXON AND P. H. MAURER, *J. Immunol.*, 74 (1954) 418.
7 F. M. DAVENPORT AND A. V. HENNESSY, *J. Exptl. Med.*, 104 (1956) 85.
8 K. E. JENSEN, F. M. DAVENPORT, A. V. HENNESSY AND T. FRANCIS, *J. Exptl. Med.*, 104 (1956) 199.
9 J. FREUND, *The Nature and Significance of the Antibody Response*, Columbia University Press, New York, N.Y., 1953, p. 46.
10 D. W. TALMAGE, H. R. BAKER AND W. AKESON, *J. Infectious Diseases*, 94 (1954) 199.
11 D. W. TALMAGE, F. J. DIXON, S. C. BUKANTZ AND G. J. DAMMIN, *J. Immunol.*, 67 (1951) 243.
12 O. SMITHIES, *Biochem. J.*, 61 (1955) 629.
13 F. J. DIXON, *J. Cellular Comp. Physiol. suppl. 1*, 50 (1957) 27.
14 D. GITLIN, H. LATTA, W. H. BATCHELOR AND C. A. JANEWAY, *J. Immunol.*, 66 (1951) 451.
15 E. FRIEDMAN, T. J. GILL, III AND P. DOTY, *J. Am. Chem. Soc.*, 83 (1961) 4050.
16 C. A. JANEWAY, *The Nature and Significance of the Antibody Response*, Columbia University Press, New York, N.Y., 1953, p. 183.
17 D. J. BUCHANAN-DAVIDSON, M. A. STAHMANN AND E. E. DELLERT, *J. Immunol.*, 83 (1959) 561.