Proc. Natl. Acad. Sci. USA
Vol. 73, No. 11, pp. 4184–4186, November 1976
Immunology

Role of antigenic structure in cell to cell cooperation

(synthetic polypeptide antigens/thymus-dependent antigen/thymus-independent antigen)

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Contributed by Michael Sela, August 25, 1976

ABSTRACT **Two synthetic polypeptides which differ only in the order of amino acids in their NH_2-terminal side chains, namely, (Tyr-Tyr-Glu-Glu)-poly(DLAla)- -poly(LLys) and (Tyr-Glu-Try-Glu)-poly(DLAla)- -poly(LLys), were found to be under different genetic control. By three different *in vivo* systems for thymus-derived cell depletion, it was demonstrated that (Tyr-Tyr-Glu-Glu)-poly(DLAla)- -poly(LLys), which represents the random poly(Tyr,Glu)-poly(DLAla)- -poly(Lys) in the pattern of immune responses and in the quality of antibodies they elicit, is thymus-dependent whereas (Tyr-Glu-Tyr-Glu)-poly(DLAla)-poly(LLys) does not require thymus-derived cell help for efficient antibody production.**

Therefore, the two ordered polypeptides which are similar chemically differ in parameters, not yet determined, which affect their capability to trigger bone marrow-derived cells.

Different types of B (bone marrow-derived) lymphocytes were reported to have different requirements for stimulation (1–3). It is also apparent that different antigens may activate antibody synthesis by dissimilar mechanisms (4). Activation of B lymphocytes usually requires helper activity from T (thymus-derived) cells as well as from a third cell type—the macrophage (5–7). A variety of antigens are able to activate B cells efficiently in the absence of T cell help (8–13). Such antigens were defined operationally, as thymus independent antigens. These immunogens can elicit an efficient immune response in T-depleted *in vivo* and *in vitro* systems (13, 14). The T-independent immunogens include polymerized flagellin (8, 9), pneumococcal polysaccharide, type S III (10), *Escherichia coli* lipopolysaccharide (11, 12), polyvinylpyrrolidone (11), and some synthetic polypeptide antigens (13). The presence of repeating antigenic determinants appears to be a necessary requirement for thymus independence (9, 12, 15). However, in addition to this feature, slow metabolism is an important characteristic of these immunogens as well (13).

In the present study, we have compared the cell requirements for eliciting an efficient antibody response to two ordered synthetic polypeptides, (Tyr-Tyr-Glu-Glu)-poly(DLAla)- -poly(LLys), abbreviated as (T-T-G-G)-A- -L, and (Tyr-Glu-Tyr-Glu)-poly(DLAla)- -poly(LLys), designated (T-G-T-G)-A- -L. These antigens are composed of the same amino acids, with the same optical configuration, and have similar molecular weights. They differ only in the order of the internal tyrosine and glutamic acid residues within the NH_2-terminal tetrapeptides (16). These polypeptides were synthesized in order to find out the nature of the major determinant of the random synthetic polypeptide poly(LTyr,LGlu)-poly(DLAla)- -poly(LLys), abbreviated as (T,G)-A- -L (17–19). It has been shown that (T-T-G-G)-A- -L represents the random (T,G)-A- -L in the pattern of immune response of inbred mouse strains, in the specificity (16, 20), the isoelectric focusing spectra (21) and the affinity of the antibodies elicited (M. Schwartz, E. Mozes, and M. Sela, unpublished data). As in the case of (T,G)-A- -L, the response potential to (T-T-G-G)-A- -L was found to be linked to the major histocompatibility (*H-2*) locus of the mouse (20). In contrast, no similarity was found between (T-G-T-G)-A- -L and the random (T,G)-A- -L as determined by the above mentioned criteria (20).

The results reported here demonstrate that, while the ordered (T-T-G-G)-A- -L is a T-dependent immunogen like the random (T,G)-A- -L, no T-B cell cooperation is needed for eliciting an immune response to the ordered (T-G-T-G)-A- -L.

MATERIALS AND METHODS

Immunogens. The immunogens used in this study were two ordered polypeptides, (T-T-G-G)-A- -L, and (T-G-T-G)-A- -L, as well as one random polymer poly(LTyr,LGlu)-poly(LPro)- -poly(LLys), abbreviated as (T,G)-Pro- -L. The synthesis, characterization and immunogenicity of these antigens have been described in earlier studies (16, 20, 22).

Animals. Mice of two inbred strains CKB and C3H.SW were obtained from the experimental animal unit of the Weizmann Institute of Science.

Transfer Experiments. Thymus, bone-marrow, and spleen cell suspensions were prepared as described (23) and injected into the tail vein (intravenously) of syngeneic recipients exposed to 800–900 roentgen from ^{60}Co gamma-irradiation. Two types of experiments were performed: (*a*) recipient mice were injected with 2 to 3×10^7 bone-marrow cells or a mixture containing 10^8 thymocytes and 2 to 3×10^7 marrow cells. (*b*) Recipients received 3×10^7 spleen cells or 3×10^7 spleen cells treated with antiserum against θ (AKR/J anti C3H/HeJ) (24) to remove T cells. In both cases, 24 hr after cell transfer each recipient was immunized intraperitoneally with 10 μg of one of the above ordered immunogens, in complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich.). The mice were bled at 11, 13, and 15 days after the transfer. The sera were individually titrated by passive microhemagglutination assay with antigen-coated sheep erythrocytes (SRBC) (25) that were previously treated with formalin (26) and tanned. In some experiments assays for hemolytic plaque forming cells (27) were done at day 11 or day 12 using fresh SRBC coated with the polypeptide (28).

Immunization of "B mice". The immune response potential of adult thymectomized, irradiated and bone marrow-reconstituted mice, "B mice" (29), was determined. These mice were

Abbreviations: B cells, bone marrow-derived cells; T cells, thymus-derived cells; (T-T-G-G)-A- -L, (Tyr-Tyr-Glu-Glu)-poly(DLAla)- -poly(LLys); (T-G-T-G)-A- -L, (Tyr-Glu-Tyr-Glu)-poly(DLAla)- -poly(LLys); (T,G)-Pro- -L, poly(LTyr,LGlu)-poly(LPro)- -poly(LLys); SRBC, sheep red blood cells.

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Table 1. The immune response of irradiated reconstituted C3H.SW mice immunized with (T-G-T-G)-A--L or (T-T-G-G)-A--L

Cells transferred	Immunization*			
	(T-T-G-G)-A--L		(T-G-T-G)-A--L	
	Exp. 1 $\log_2$HA†	Exp. 2 PFC‡	Exp. 1 $\log_2$HA†	Exp. 2 PFC‡
Bone marrow (2×10^7)	2.6 ± 0.7	1860 ± 370	3.5 ± 0.5	3610 ± 1130
Bone marrow (2×10^7) + thymocytes (10^8)	5.3 ± 0.6	4770 ± 590	3.8 ± 0.9	2840 ± 240

* Each group contains five animals.
† Microhemagglutination (HA) performed at day 13.
‡ Determination of number of plaque forming cells (PFC)/spleen at day 11.

immunized three weeks after the reconstitution with 10 μg of (T-G-T-G)-A- -L or (T,G)-Pro- -L, given intradermally into the hind foot pads.

RESULTS

The requirements for thymocytes in the immune response to (T-G-T-G)-A- -L and (T-T-G-G)-A- -L

C3H.SW mice, 10 weeks of age, were irradiated and injected with bone marrow or bone marrow together with thymocytes, and then immunized with (T-T-G-G)-A- -L or (T-G-T-G)-A- -L. As can be seen in Table 1, production of antibodies specific to (T-G-T-G)-A- -L as determined by either plaque forming cell assay or by microhemagglutination could be achieved without thymocytes. Furthermore, addition of thymocytes did not enhance the response to (T-G-T-G)-A- -L. In contrast, the antibody levels produced to (T-T-G-G)-A- -L in the absence of thymocytes was low and increased significantly with the addition of thymocytes.

The effect of T cell depletion from spleens on the immune response to (T-G-T-G)-A- -L

The immune response to (T-G-T-G)-A- -L of recipients of either normal spleen cells or spleen cells after anti-θ treatment was compared. As shown in Table 2, the antibody responses to (T-G-T-G)-A- -L of mice injected with either spleen cells or antiserum against θ treated spleen cells were similar. Addition of thymocytes to spleens treated with antiserum against θ did not enhance the response.

Immune response of "B mice" to (T-G-T-G)-A- -L

To confirm the T-independence of (T-G-T-G)-A- -L, we determined the immune response of CKB "B mice" to (T-G-T-G)-A- -L. In comparison, the immune response of "B mice" to the thymus-dependent antigen, (T,G)-Pro- -L, was checked. Three days after the last bleeding, the animals used in this experiment were immunized with sheep red blood cells (SRBC), to verify the T cell depletion in the "B mice." The secondary antibody responses represented in Table 3 indicate that the immune response potential of "B mice" to (T-G-T-G)-A- -L was as high as of normal mice which possessed both T and B cells. In contrast, such "b mice" did not produce antibodies to the T-dependent synthetic antigen (T,G)-Pro- -L (25), the response to which was high in normal CKB mice. As can be seen, the "B mice" produced a negligible antibody response to SRBC, whereas the normal CKB mice responded with high antibody titers.

Table 2. The immune response to (T-G-T-G)-A--L of irradiated reconstituted CKB mice

Cells transferred	Log_2HA*
Intact mice	3.5 ± 0.40
Spleen cells (3×10^7)	4.0 ± 0.34
Spleen cells treated with antiserum against θ (2×10^7)	3.6 ± 0.23
Spleen cells treated with antiserum against θ (2×10^7) + thymocytes (1×10^8)	2.7 ± 0.22

* Average of $\log_2$ hemagglutination of 5–6 animals in each group. The titers of control sera from irradiated, spleen repopulated, nonimmunized mice was lower than 2.

DISCUSSION

The structure of an antigen has a marked effect on its capacity to stimulate B cells (4). Even though the chemical properties of antigen determine the requirement for cell to cell cooperation in eliciting an immune response, the mechanism of the triggering of B cells has not been yet established. It appears that several classes of T-independent antigens exist (4). The common denominator for all the T-independent antigens yet studied is the presence of repeating antigenic determinants. However, additional features, such as slow metabolism, have been also reported to be required (4).

The two ordered synthetic polypeptides, (T-T-G-G)-A- -L and (T-G-T-G)-A- -L, appeared to be good candidates for studying the relationship between the chemical nature of antigens and the need for cell to cell cooperation in eliciting an immune response. These two antigens are very similar in their chemical structure but, nevertheless, the immune responses towards them were found to be under different genetic controls (20).

This study (Table 1) demonstrated that (T-T-G-G)-A- -L is a T-dependent immunogen as expected from its similarity to the random (T,G)-A- -L (30). In contrast, (T-G-T-G)-A- -L can activate B cells to elicit antibodies without the help of thymocytes or T-cells (Tables 1–3). The T-independence of (T-G-T-G)-A- -L was confirmed in a parallel study performed with newborn mice which produced antibodies to this ordered immunogen but not to (T-T-G-G)-A- -L (B. Hardy, M. Schwartz, and E. Mozes, unpublished data). Newborn mice at their first week of life lack functional macrophages and T cells, and therefore are not capable of responding to T-dependent immunogens. However, they were previously shown to produce antibodies to a T-independent synthetic polypeptide (31).

The two ordered polypeptides investigated are very similar chemically as both of them possess repeating antigenic determinants, and they are not expected to differ in their rate of metabolism since they are composed of only L-amino acids. Thus, by the criteria mentioned above, they would be expected to possess similar characteristics concerning their need for T cell cooperation in order to induce against them an efficient im-

Table 3. Immune response of CKB "B mice" to (T-G-T-G)-A--L

Animals	Log_2 hemagglutination*			
	(T,G)-Pro--L†	(T-G-T-G)-A--L†	(T-G-T-G)-A--L‡	SRBC§
CKB "B mice"	1.6 ± 1.0	3.9 ± 0.9	4.5 ± 0.28	3.3 ± 0.35
CKB intact mice	5.7 ± 0.87	3.8 ± 0.85	4.4 ± 0.48	6.8 ± 0.32

* Mice were injected with 10 μg of antigen with complete Freund's adjuvant and boosted with the same dose of antigen in aqueous solution.
† Sera obtained 11 days after booster injection.
‡ Sera obtained 14 days after booster injection.
§ Titers of the same mice immunized 17 days after booster injection with 10^8 SRBC and bled 5 days later.

mune response. They should differ in other parameters which affect their capability to trigger B cells. One such parameter may relate to the physicochemical nature of the two polymers, such as their three-dimensional structure. Further studies on the differences between (T-G-T-G)-A- -L and (T-T-G-G)-A- -L may lead to a general model for T-independence and a better understanding of the mechanism of B cell stimulation.

This research was supported in part by Grant 1R01 AI 11405-04 from the National Institutes of Health, USPHS. R.J.H. is a recipient of a long-term EMBO Fellowship.

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