EFFECT OF SYNTHETIC POLYELECTROLYTES ON COOPERATION BETWEEN T AND B CELLS DURING IMMUNIZATION OF MICE OF DIFFERENT GENOTYPES WITH ARTIFICIAL ANTIGENS (T,G)-A-L

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UDC 612.112.94.017.1-063

KEY WORDS: synthetic polyelectrolytes; T and B cells; cellular cooperation; IR-genes; genotype.

Synthetic polyelectrolytes [polyacrylic acid (PAA)], weak polybases [poly-4-vinylpyridine (PVP), poly-2-methyl-5-vinylpyridine], and alkyl derivatives of these polybases have a strong stimulating action on the immune response to corpuscular and soluble thymus-dependent antigens of different origin [1, 3-8].

As special investigations have shown, their immunostimulating action is based on potentiation of individual stages of immunogenesis: proliferation and migration of hematopoietic stem cells, migration and cooperation of T and B cells in the immune response [3, 5, 6], and ability to partly take over the helper function of T lymphocytes [5, 7].

Since in all the investigations cited above the effect of synthetic polyelectrolytes was studied on the immune response to complex antigens (erythrocytes, proteins, hapten–protein conjugates) which is under polygenic control, in the present investigation it was decided to study the effect of PVP and PAA on the immune response to a synthetic polypeptide antigen (T,G)-A-L, which is controlled by the Ir-IA gene.

Ir-IA is an autosomal dominant gene, linked with the major histocompatibility complex (H-2), and controls the immune response to multichain branched polypeptides (T,G)-A-L, (H,G)-A-L, and (P,G)-A-L [9, 11, 12]. Mice with the H-2^b haplotypes are highly responsive to (T,G)-A-L, those of haplotype H-2^d are moderately responsive, and those of haplotypes H-2^k, H-2^q, H-2^r, H-2^s, and H-2^w, are weakly responsive or unresponsive [9, 10, 12, 14]. The greatest differences between the responsive and unresponsive phenotypes are exhibited on reimmunization at the level of synthesis of IgG antibodies.

The existence of individuals with genetically determined low responses to certain antigens makes the problem of phenotypical correction, i.e., conversion of weakly to strongly reactive phenotypes, extremely important [2].

It has been shown in the system of adoptive transfer [15] that the IgM response to (T,G)-A-L also is controlled by the Ir-IA gene. The writers have used this experimental model to study the effect of PVP and PAA on the immune response controlled by the Ir-IA gene. Besides a quantitative analysis of the effect of these synthetic polyelectrolytes at the level of the immune response controlled by one gene, this model also enables the effect of PVP and PAA on cooperation between T and B lymphocytes to be studied. This is particularly important, for sites of expression of the Ir-IA gene are T lymphocytes and their ability to interact with B lymphocytes.

EXPERIMENTAL METHOD

Experiments were carried out on mice of strains CBA (H-2^k), C57BL/6 (H-2^b), (CBA × C57BL/6)F_1 [(H-$2^{k/b}$) and BALB/c (H-2^d) weighing 20-22 g, obtained from the Stolbovaya Laboratory Animal Nursery, Academy of Medical Sciences of the USSR.

(T,G)-A-L with a molecular weight of 282,000 (from Miles Laboratories Inc., USA), in a dose of 10 μg per mouse, was used as the antigen.

Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 590-592, May, 1981. Original article submitted August 21, 1980.

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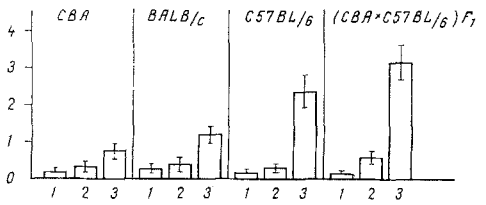

Fig. 1. Cooperative interaction between T and B cells from mice of different strains during induction of primary immune response to (T,G)-A-L in adoptive cell transfer system. Ordinate, here and in Figs. 2 and 3: number of AFC per spleen ($\times 10^3$). 1) thymocytes; 2) bone marrow cells; 3) thymocytes + bone marrow cells. Averaged results of three experiments are given; altogether 12-16 mice of each genotype were used.

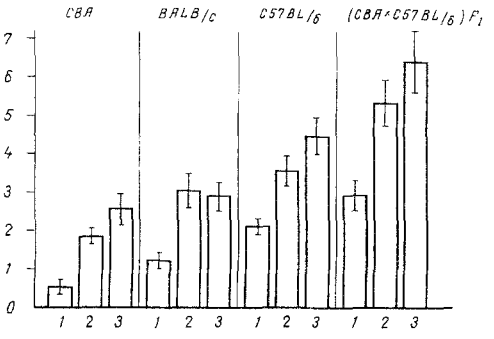

Fig. 2

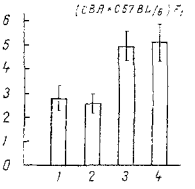

Fig. 3

Fig. 2. Effect of PVP and PAA on cooperative interaction between T and B cells in immune response to (T,G)-A-L. 1) Thymocytes + bone marrow cells; 2) thymocytes + bone marrow cells + PVP; 3) thymocytes + bone marrow cells + PAA. Averaged results of three experiments shown; 9-12 mice of each genotype were used.

Fig. 3. Activation of thymus cells by (T,G)-A-L in an in vivo system in the presence of PVP and PAA. 1) Thymus + bone marrow cells; 2) ATC + bone marrow cells; 3) ATC_{PAA} + bone marrow cells; 4) ATC_{PVP} + bone marrow cells. Legend: ATC) activated T cells in the presence of (T,G)-A-L; ATC_{PAA}) activated T cells in the presence of (T,G)-A-L + PAA; ATC_{PVP}) activated T cells in the presence of (T,G)-A-L and PVP. Results of three experiments shown; 16-18 mice were used in each group.

To study the effect of synthetic polyelectrolytes on the cooperative effect of T–B interaction, syngeneic bone marrow (10^7) and thymus (4×10^7) cells mixed with (T,G)-A-L were injected intravenously into lethally irradiated ($LD_{100/14}$) recipients. After injection of the cells into the recipients, PVP or PAA in aqueous solution was injected into the recipients in a dose of 50 mg/kg body weight. The number of IgM-secreting antibody-forming cells (AFC) in the recipients' spleen was determined 10-12 days after transplantation of the cells by a modified Jerne's method, using sheep's red blood cells loaded with (T,G)-A-L [15]. The reagent used was $CrCl_3 \cdot 6H_2O$ (from Merck, West Germany, mol. wt. 266.45).

To obtain activated T cells, lethally irradiated recipients were given an intravenous injection of syngeneic thymocytes in a dose of 50×10^6–60×10^6 together with (T,G)-A-L and PVP (intravenously) or together with (T,G)-A-L and Freund's complete adjuvant (intraperitoneally). Spleen cells were obtained 6 days after injection of the cells and immunization and, mixed with bone marrow cells, they were injected into lethally irradiated syngeneic recipients together with (T,G)-A-L.

EXPERIMENTAL RESULTS

The results of a study of interaction between T and B lymphocytes during induction of the immune response to (T,G)-A-L in an adoptive transfer system are given in Fig. 1. It will be clear from Fig. 1 that on injection of bone marrow cells (B lymphocytes) together with (T,G)-A-L very few AFC synthesizing antibodies against (T,G)-A-L were formed. A few AFC against (T,G)-A-L also were observed after transplantation of thymocytes (T lymphocytes).

Injection of a mixture of bone marrow cells with thymocytes caused accumulation of AFC to a number eight to ten times greater than the total number of AFC formed in response to separate injection of B and T lymphocytes. These experiments determine the need for cooperation between T and B lymphocytes for induction of the immune response to (T,G)-A-L, in good agreement with data published previously [14, 15].

As Fig. 1 shows, interlinear differences during induction of the primary immune response to (T,G)-A-L were observed in the adoptive transfer system of T and B cells, i.e., at the level of formation of IgM-AFC. For instance, C57BL/6 mice were highly responsive, CBA gave only a weak response, and BALB/c mice were moderately responsive. The ability of (CBA × C57BL/6)F_1 hybrids to produce IgM-AFC in the adoptive transfer system of D and B cells of the highly responsive type is inherited as a dominant character.

After injection of a mixture of bone marrow and thymus cells together with PAA into lethally irradiated syngeneic recipients, twice or three times as many AFC against (T,G)-A-L accumulated in their spleens as in recipients receiving a mixture of T and B lymphocytes only. A similar result also was observed when PVP was injected together with T and B lymphocytes (Fig. 2).

In response to injection of PAA or PVP together with bone marrow and spleen cells, potentiation of cooperative T–B interaction was thus observed during induction of the immune response to (T,G)-A-L. It must be pointed out that cooperative interaction between T and B lymphocytes during the immune response to (T,G)-A-L was enhanced by two or three under the influence of polyelectrolytes in mice of highly responsive genotypes C57BL/6 and (CBA × C57BL/6)F_1, and by three to five times in the weakly responding CBA strain. As a result, the height of the immune response in weakly responding mice treated with the polyelectrolyte was raised up to the level of the response in the highly responsive individuals and not treated with the polyelectrolyte, i.e., phenotypical correction of the immune response to (T,G)-A-L took place.

Results showing the effect of PVP on the process of activation of the T cells are given in Fig. 3. In response to injection of bone marrow cells together with T cells activated by (T,G)-A-L in the presence of PVP of Freund's complete adjuvant into irradiated recipients, from 2 to 2.5 times more AFC were formed in the recipients' spleen in response to immunization with (T,G)-A-L than after injection of bone marrow cells and thymocytes activated by (T,G)-A-L without the additional injection of the polyelectrolyte or adjuvant. In other words, PVP potentiates the process of activation or "training" of the T-helpers by synthetic antigens (T,G)-A-L.

These experiments thus showed that differences in the immune response to (T,G)-A-L at the level of synthesis and secretion of IgM antibodies, controlled by the Ir-IA gene, are manifested in a system of adoptive transfer of cooperating T cells.

Injection of synthetic polyelectrolytes into recipients of T and B cells potentiates the processes of activation of T helpers and of T–B cooperation during induction of the immune response. Under these circumstances, potentiation of the process of T–B cooperation was expressed more strongly in mice of a genotype with low response to (T,G)-A-L. As the result of this the low immune response controlled by the Ir-IA gene is corrected (stimulated). The results demonstrate for the first time that an immune response controlled by a concrete Ir gene can be corrected by synthetic polyelectrolytes. The mechanism of enhancement of the immune response is linked with intensification of T–B cooperation.

LITERATURE CITED

1. V. A. Kabanov, M. I. Mustafaev, A. Sh. Norimov, et al., Dokl. Akad. Nauk SSSR, 243, 1330 (1978).
2. R. V. Petrov, Immunology and Immunogenetics [in Russian], Moscow (1976).
3. R. V. Petrov, A. N. Gvozdetskii, A. A. Gorokhov, et al., Zh. Mikrobiol., No. 11, 121 (1974).
4. R. V. Petrov and R. M. Khaitov, in: Progress in Science and Technology. Series Immunology [in Russian], Vol. 7, Moscow (1978), p. 223.
5. R. V. Petrov, R. M. Khaitov, E. V. Kozhinova, et al., Tsitologiya, 17, No. 3, 321 (1975).
6. R. V. Petrov, R. M. Khaitov, E. V. Kozhinova, et al., Tsitologiya, No. 3, 321 (1975).

7. R. M. Khaitov, Med. Ref. Zh., Section 21, No. 3, 9 (1976).
8. R. M. Khaitov and A. A. Batyrbekov, Byull. Éksp. Biol. Med., No. 5, 582 (1976).
9. B. Benacerraf and H. O. McDevitt, Science, 175, 273 (1972).
10. L. Lichtenberg, E. Mozes, G. M. Shearer, et al., Eur. J. Immunol., 430 (1974).
11. H. O. McDevitt and M. Sela, J. Exp. Med., 126, 969 (1967).
12. H. O. McDevitt, K. B. Bechtol, F. C. Grumet, et al., in: Progress in Immunology, ed. B. Amos, New York (1971), p. 495.
13. E. Mozes, G. M. Shearer, M. Sela, et al., J. Immunol., 3, 439 (1973).
14. A. J. Munro and M. J. Taussig, Nature, 256, 103 (1975).
15. M. J. Taussig, E. Mozes, and R. Isac, J. Exp. Med., 140, 301 (1974).

IMMUNE INTERFERON FORMATION AND BLAST TRANSFORMATION IN STIMULATED HUMAN LYMPHOCYTE CULTURES

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UDC 612.112.94.017.1-063

KEY WORDS: immune interferon; blast transformation of lymphocytes.

There has been a steady rise in recent years in interest in the regulatory effect of interferon (IN) on the immune response, described by many workers [4, 8, 9, 12]. Immune IN, or type II IN, is nowadays regarded as a mediator secreted by lymphocytes in response to their stimulation [6, 14, 15].

The study of the individual features of immune IN production under the influence of mitogens and antigens by lymphocytes from people in different age groups is interesting. In the literature on this subject there are only a few, somewhat contradictory communications [5, 13].

The aim of the present investigation was to study production of IN and the dynamics of its formation in lymphocyte cultures (LC), obtained from children and adults and stimulated by phytohemagglutinin (PHA) and dried, purified tuberculin (PPD), and to study the relations between these processes and blast transformation of lymphocytes (BTL).

EXPERIMENTAL METHOD

Blood for LC was obtained from seven adults (aged 40-50 years) and 24 children (aged 5-7 years). IN formation was studied in 82 LC (52 from children and 30 from adults). The method of obtaining LC and of evaluating BTL was described in detail by the writers previously [2]. IN was determined in the supernatant of 3-day cultures stimulated by PHA (PHA-P, from Difco, USA) in a dose of 10 μg/ml, and 6-day LC stimulated by PPD (from Leningrad Institute of Vaccines and Sera) in a dose of 100 μg/ml. As the control, IN was determined in unstimulated LC from the children and adults under investigation, and also in nutrient medium with the addition of PHA and PPD, incubated for three and six days.

IN was determined by the method based on delay of the cytopathic effect produced in primary trypsinized human embryonic cultures by 100 CPD_{50} of vesicular stomatitis virus, Indiana strain.

EXPERIMENTAL RESULTS

IN production was studied in 35 LC stimulated by PHA and in 20 LC stimulated by PPD. During stimulation by PHA most indices of BTL in LC varied from 60 to 90% which, as was shown previously [2], is normal for clinically healthy persons. However, during a normal proliferative response to PHA, IN formation was absent in LC from 16 subjects (15 children and one adult). The negative response in six children was confirmed on retesting. This may be due both to low production of IN by children's leukocytes [3] or to the fact that the IN concentration was below the threshold of sensitivity of the method used to determine it. In the

Tallin Research Institute of Epidemiology and Microbiology. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 592-594, May, 1981. Original article submitted January 18, 1981.

