CYTOTOXIC ACTIVITY OF MOUSE LYMPHOCYTES SENSITIZED BY CROSS-REACTING MICROBIAL ANTIGENS

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Lymphocytes of CC57Br, CBA, and C3H mice obtained on the 5th to 17th days after immunization with streptococcal group C and Candida albicans vaccines have a cytotoxic action on cultures of L-cells. The cytotoxic action of the sensitized lymphocytes was suppressed by incubation for 8 h with disintegrated group C streptococcal vaccine and with skin extract of C3H mice and also after the addition of mouse antistreptotoccal and antiskin sera of CC57Br and CBA mice to the culture of L-cells.

The presence of antigens cross-reacting with mammalian tissues in certain microorganisms (Streptococcus, Candida albicans) may lead to sensitization accompanied by damage to autologous tissues [2, 4, 5, 7, 8] and acceleration of rejection of skin allografts [9]. The development of hypersensitivity of delayed type is probably an important factor in the induction of tissue damage under these circumstances [5]. The discovery of cytotoxic activity in the lymphocytes of animals sensitized by cross-reacting microbial antigens is important for the elucidation of the mechanism of development of the immunopathological process

In this investigation the cytotoxic activity of lymphocytes of various lines of mice immunized with microbial antigens with an affinity of mouse tissues was studied against cultures of mouse fibroblasts.

EXPERIMENTAL METHOD

Female mice of lines C57Br ($H\text{-}2^b$), CBA ($H\text{-}2^k$), and C3H ($H\text{-}2^k$) weighing 16-18 g were used as the experimental animals. The mice were immunized subcutaneously with group C streptococcal, C. albicans, and Escherichia coli vaccines three times at intervals of 24 h ($10^7 - 10^8 - 1.5 \times 10^8$ bacterial cells per injection). At certain times intervals after vaccination the mice were exsanguinated and the lymph glands and spleen removed with aseptic precautions. Suspensions of lymphocytes were prepared and cultures of L-cells (fibroblasts of C3H mice) were grown by the usual method [6]. Suspensions of lymphocytes in which the proportion of dead cells before their addition to the culture or after incubation with antigen did not exceed 10% as shown by staining with trypan blue, were used in the experiment. On the day of the experiment the L-cells were washed three times with medium No. 199. The lymphocytes were resuspended in the same medium up to a concentration of 10^7 cells/ml, and 1 ml was added to the culture of L-cells and incubated for 3 days at 37°C. The number of living L-cells in 1 ml of medium in the tube and the cytotoxic index (CI) of the sensitized lymphocytes were determined by the method of Brondz [1]. At each time 90 tubes in each of the experimental and control groups were studied.

In the experiments in which the cytotoxic action (CA) of the lymphocytes was blocked, group C streptococcal vaccine and E. coli vaccine containing 10^{10} bacterial cells/ml, disintegrated with salt, and saline extracts of C3H mouse skin with a protein content of 30 mg/ml were used. The target cells were blocked by immune sera of CC57Br, CBA, and C3H mice against group C streptococcal vaccine with a titer in the complement fixation tests (CFT) of 1:54 and also with the serum of CC57Br and CBA mice against skin extract of C3H mice with a titer in the CFT of 1:40 and 1:20, respectively. The antigen and the normal

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TABLE 1. Effect of Streptococcal Antigens and Skin Extract and of Mouse Immune Sera against These Antigens on Cytotoxic Action of Lymphocytes of Mouse Immunized with Group C Streptococcal Vaccine

Group tested	Sensitized mouse lymphocytes					
	CC57Br		CBA		C3H	
	no. of living cells, × 100	CI	no. of living cells, × 100	CI	no. of living cells, × 100	CI
Sensitized lymphocytes treated with: physiological saline	56,2±12,2	0,97	359,0±64,0	0,88	376,4±58,4	0,85
Disintegrated group C streptococcal vaccine	209,6±21,0 $P<0,001$	0,9	718,1±21,3 $P<0,001$	0,76	1001,1±83,3 $P<0,001$	0,70
Skin extract of C3H mice	131,1±17,2 $P<0,001$	0,93	538,7±17,8 $P<0,01$	0,8	840,1±56,6 $P<0,001$	0,75
Disintegrated E. coli vaccine	66,0±18,3 $P>0,1$	0,97	363,3±37,1 $P>0,1$	0,87	380,8±43,1 $P>0,1$	0,85
L-cells treated with: normal mouse serum	53,6±12,3	0,97	293,6±27,3	0,89	440,5±46,6	0,86
Antistreptococcal serum of CC57Br mice	304,3±41,7 $P<0,001$	0,8	1291,2±58,6 $P<0,001$	0,55	1552,5±192,1 $P<0,001$	0,51
Antistreptococcal serum of CBA mice	65,4±14,8 $P>0,05$	0,96	760,8±10,8 $P<0,001$	0,73	728,5±77,0 $P<0,001$	0,77
Antistreptococcal serum of C3H mice	62,8±10,6 $P>0,1$	0,96	351,5±11,6 $P>0,05$	0,88	448,8±26,3 $P>0,1$	0,86
Antiskin serum of CC57Br mice	564,7±20,8 $P<0,001$	0,73	1270,1±42,6 $P<0,001$	0,55	1510,3±127,7 $P<0,001$	0,52
Antiskin of normal mice	168,3±10,6 $P<0,001$	0,92	693,8±29,3 $P<0,001$	0,73	810,5±53,3 $P<0,001$	0,74
Lymphocytes of normal mice	2090,0±10,6	—	2925,0±149,1	—	3181,2±141,3	—

*Number of living L-cells, × 100.

and immune mouse sera were added to the experimental and control samples in volumes of 0.2 ml to 1 ml of culture medium. Lymphocytes were incubated with antigen at 37°C for 30 min or 4 or 8 h, and the L-cells were incubated with serum for 30 min at room temperature and then washed three times with medium No. 199.

EXPERIMENTAL RESULTS

After the addition of lymphocytes of CC57Br, CBA, and C3H mice to the tissue culture, destruction of the tissue culture was observed if the lymphocytes had been obtained between the fifth and seventeenth days after vaccination of the animals with group C streptococcal vaccine or C. albicans. The lymphocytes of these lines of mice, immunized with E. coli vaccine, had no CA of the tissue culture. Lymphocytes of mice immunized with streptococcal vaccine and C. albicans possessed maximal cytotoxic activity on the 7th day after vaccination. The intensity of CA of the mouse lymphocytes was about the same after injection of streptococcal or C. albicans vaccines. However, some difference was found in the activity of the sensitized lympocytes between the various lines of mice. For instance, lymphocytes obtained on the 7th day after vaccination with streptococcal vaccine or C. albicans from CC57Br mice had a greater CA on the culture of L-cells (2,800 ± 730 and 1,560 ± 360 living L-cells in the experimental series and 216,510 ± 13,270 L-cells in the control; CI 0.98 and 0.99; $P<0.001$) than lymphocytes of CBA mice (32,000 ± 1760 and 23,500 ± 2150 living L-cells in the experiment and 286,570 ± 12,160 L-cells in the control; CI 0.88 and 0.91; $P<0.001$) and C3H (43,820 ± 1630 and 36,750 ± 2070 living L-cells in the experiment and 319,710 ± 15,230 L-cells in the control; CI 0.86 and 0.88; $P<0.001$).

When lymphocytes of CC57Br, CBA, and C3H mice were obtained on the 7th day after immunization with streptococcal vaccine and skin extract of C3H mice or with disintegrated streptococcal vaccine, a decrease in their cytotoxic activity was observed only after incubation for 8 h with the antigen. Under the circumstances the streptococcal antigen caused more marked suppression of CA of the sensitized lymphocytes than skin extract of C3H mice (Table 1). Treatment of lymphocytes of CC57Br, CBA, and C3H mice immunized with streptococcal vaccine or disintegrated E. coli vaccine did not affect the cytotoxic reaction. Incubation of normal lymphocytes with microbial and tissue antigens did not change the reaction of the lymphocytes to the tissue culture.

A decrease in CA of lymphocytes obtained from CC57Br, CBA, and C3H mice 7 days after immunization with streptococcal vaccine also was observed after preliminary incubation of the L-cells with antistreptococcal and antiskin sera of CC57Br and CBA mice, the antiskin serum being more effective than the antistreptococcal serum (Table 1). In both cases the blocking activity of the sera of the CC57Br mice was greater than that of the sera of CBA mice. Antistreptococcal serum of C3H mice was inactive in suppressing CA of the sensitized lymphocytes. After treatment of the culture of L-cells with the sera of normal mice no blocking of CA of the lymphocytes of the sensitized mice was found. The addition of lymphocytes of unimmunized mice to L-cells preincubated with antitissue or antimicrobial sera had no effect on their cytotoxic activity.

These results show that lymphocytes of mice immunized with group C streptococcal and C. albicans vaccines can destroy a culture of L-cells. The observed injury to the tissue culture is probably not attributable to the direct toxic action of microorganisms remaining in the lymphoid tissue after injection on the L-cells. For instance, lymphocytes obtained from mice not earlier than 5 days after vaccination, when the number of microorganisms in the lymphoid tissue is much less than on the fifth day after immunization, possess CA. The possibility that the tissue culture may be damaged by toxic factors secreted by the lymphocytes before their death as a result of possible contact in the culture with living microorganisms was ruled out by strict observance of asepsis when the lymphoid organs were obtained and by careful microbiological checks.

The possibility remains that the CA of lymphocytes of the vaccinated mice was immunological in nature and was due to sensitization of the lymphocytes to streptococcal and C. albicans antigens related to the antigens of the tissue culture. This was confirmed by the experiments with crossed blocking of CA of the lymphocytes by means of streptococcal antigens and skin antigens of C3H mice and also by immune sera obtained against these antigens. That part of the antigens to which sensitization arises in the lymphocytes on vaccination is probably controlled by H-2^k histocompatibility locus. This may explain the maximal cytotoxic activity lymphocytes of sensitized CC57Br mice, with an H-2^b histocompatibility locus and the lower activity of CBA and C3H mice, with the H-2^k histocompatibility locus, like cultures of L-cells.

The fact that the microbial antigen possessed the greatest activity in blocking CA of the sensitized lymphocytes, and that antitissue serum possessed the greatest activity when immune sera were used may be attributed to differences in the mechanism of inhibition of CA of the lymphocytes [1]. In the first case lymphocyte activity was most completely neutralized by antigen inducing sensitization in the mice. In the second case the receptors of the L-cells would be completely blocked and sorption of lymphocytes on them would be prevented by antiserum against skin antigen, which probably has a higher content of antibodies against the tissue culture than the antistreptococcal serum.

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