THE USE OF CROSS-REACTING MICROBIAL ANTIGENS TO ABOLISH TOLERANCE PRODUCED TO TRANSPLANTATION MOUSE ANTIGEN

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Immunization of CC57BR mice tolerant to Candida albicans antigens and to related C3H mouse transplantation antigens and to group A (type I) Streptococcus containing antigens cross-reacting with the donor's tissues partially abolished tolerance to C3H mouse alloantigens. This was manifested as shortening of the survival of skin allografts and an increase in the rate of elimination of ^{51}Cr-labeled donor's lymphocytes from the lymph nodes and spleen. The immune response to C. albicans antigens was not restored.

KEY WORDS: tolerance; transplantation; cross-reacting microbial antigens.

The presence of antigens related to tissue antigens in microorganisms (Streptococcus, Candida albicans) suggests that they play a role in the induction of some pathological processes in subjects sensitized by them [1,2,5]. A lesion may arise in the tissues under these circumstances through the abolition of immunological tolerance to the host's related autoantigens by cross-reacting microbial antigens [2].

The possibility of abolition of artificially created immunological tolerance to certain serum protein antigens by immunization with related antigens has been demonstrated by Weigle [6]. However, there is no experimental evidence to confirm that tolerance to tissue antigens can be abolished by cross-reacting microbial antigens.

In this investigation a model of tolerance to mouse transplantation antigens induced artificially by cross-reacting antigens of Candida albicans was used to investigate the possibility of its abolition by injection of cross-reacting antigens from another microbial species (streptococcus).

EXPERIMENTAL METHOD

Experiments were carried out on CC57BR (H-2^b) recipient mice and C3H (H-2^k) donor mice. Killed vaccines of C. albicans and group A Streptococcus (type I) and also vaccine of Escherichia coli not containing cross-reacting antigens, were used as microbial antigens containing antigens related to mouse tissues. Tolerance to C. albicans and to the related C3H mouse transplantation antigens was produced in CC57BR mice by injection of the vaccine in a dose of 10^{10} microbial cells per injected intramuscularly in three doses during the first 3 days after birth and on three further occasions subsequently at weekly intervals. After 1 month tolerance to C. albicans antigen and to cross-reacting transplantation antigen was determined in the CC57BR mice: 1) by the absence of antibody formation after triple immunization of the mice with C. albicans; 2) by the increase in survival of allografts of C3H mouse skin; 3) by the rate of elimination of ^{51}Cr-labeled C. albicans cells and C3H mouse lymphocytes from the mice.

In the experiments to study abolition of immunological tolerance in animals, experimental and control CC57BR mice of the same age were immunized with 10^{10} cells of C. albicans, Streptococcus, and E. coli, by injecting them in 0.3 ml Freund's incomplete adjuvant either once subcutaneously or three times intramuscularly at intervals of 5 days. Fourteen days after immunization of the CC57BR mice skin grafts were transplanted from C3H mice and the length of their survival was noted, or $5 \cdot 10^6$ C3H mouse lymphocytes containing

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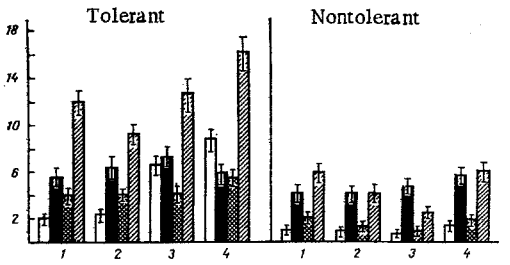

Fig. 1. Distribution of radioactivity in mesenteric lymph nodes and spleen of tolerant and nontolerant CC57BR mice immunized with microbial antigens after injection of ^{51}Cr-labeled C3H mouse lymphocytes and C. albicans cells. Unshaded and black columns show levels of radioactivity of radioactivity in lymph nodes after injection of labeled lymphocytes and C. albicans cells respectively; cross-hatched and obliquely shaded columns show levels of radioactivity in spleen after injection of labeled lymphocytes and C. albicans cells respectively. 1) Mice immunized with Streptococcus, 2) with Streptococcus and C. albicans, 3) with C. albicans, 4) unimmunized mice. Ordinate, level of radioactivity (x 10^3 counts/min).

transplantation antigens or 10^9 C. albicans cells labeled with ^{51}Cr [3] were injected intravenously, and the quantity of radioactive label in the spleen and mesenteric lymph nodes was determined 24 h later by Bainbridge's method [4]. Each experimental and control group for the experiments with the transplantation test contained at least 20 mice. To study the distribution of radioactive label the organs for investigation were obtained individually from five mice of each group. Some of the immunized mice were killed at this period to obtain serum. Antibodies against microbial antigens were determined in the microbial agglutination reaction with heat-killed cells of C. albicans, group A (type I) Streptococcus, and E. coli. Antibodies against C3H mouse antigens were revealed by the saline leuko- and hemagglutination tests. Eight samples of sera (each sample being a mixture of sera from three mice) of the experimental and control groups were investigated.

EXPERIMENTAL RESULTS

Six injections of C. albicans vaccine into CC57BR mice starting within a few days of birth induced a state of immunological tolerance in the animals both to the microbial antigen injected and also to related tissue antigens of C3H mice. The formation of agglutinins against C. albicans in response to subsequent triple immunization with C. albicans vaccine was inhibited in the tolerant mice. The survival of skin allografts in the tolerant recipients was lengthened to 47.6±4.3 days ($P < 0.001$) compared with 9.6±0.8 days in the control. In CC57BR mice tolerant to C. albicans antigens, ^{51}Cr-labeled C. albicans cells and C3H mouse lymphocytes injected were eliminated 2-5 times more slowly from the spleen and lymph nodes ($P < 0.001$) than from intact animals (Fig. 1). The specificity of the resulting tolerance was confirmed by preservation of the normal immune response to triple intraperitoneal injection of E. coli vaccine or a single intravenous injection of $5 \cdot 10^8$ sheep's red cells.

If CC57BR mice tolerant to C. albicans were immunized by three intraperitoneal injections of Streptococcus, antibodies against Streptococcus appeared in a titer of 1:64-1:128, and also against red cells and lymphocytes of C3H mice in titers of 1:4-1:8, just as after immunization of nontolerant mice. No antibodies against C. albicans were found. On subsequent triple immunization of these mice 20 days later with C. albicans no antibodies against C. albicans and C3H mouse tissue antigens could be found. Conversely, in the control group of nontolerant mice immunized in the same way, antibodies against C. albicans were found in titers of 1:64-1:128, against Streptococcus in titers of 1:8-1:16, and against C3H mouse red cells and lymphocytes in titers of 1:16-1:32. In the case of triple immunization of tolerant mice with E. coli vaccine antibodies against E. coli antigens only were detected in the serum. Subsequent triple immunization of tolerant animals of this group with C. albicans likewise did not lead to the appearance of antibodies in the serum against antigens of C. albicans or C3H mouse red cells and lymphocytes.

A single subcutaneous immunization of tolerant CC57BR mice with C. albicans had no significant effect on the survival of skin grafts. If streptococcal vaccine was injected into the tolerant mice, the survival period of the allografts was reduced in these mice compared with unimmunized tolerant animals (down to 13.1±0.6 days; $P < 0.001$). The period of survival of allografts in these mice still remained shortened 20 days after a single immunization with C. albicans. In the nontolerant mice a single subcutaneous immunization with both Streptococcus and C. albicans shortened the survival of the skin allografts to between 6.2±0.5 and 6.3±0.5 days from 9.6±0.8 days in the control ($P < 0.001$). A similar decrease in the survival of skin allografts in nontolerant mice also was discovered after immunization of the mice with Streptococcus and C. albicans 20 days later. Immunization of tolerant and intact mice with E. coli vaccine did not affect the survival period of the skin allografts.

Simultaneous subcutaneous immunization of tolerant mice with C. albicans, unlike immunization of the nontolerant mice, did not accelerate the elimination of labeled lymphocytes and of C. albicans cells from the lymphoid organs (Fig. 1). After a single injection of Streptococcus into the tolerant animals the elimination of labeled lymphocytes only was accelerated (compared with the group of nonimmunized tolerant animals) from the lymph nodes, just as in the case of a single immunization of the mice with C. albicans 20 days later (Fig. 1). Under these circumstances the rate of elimination of the labeled lymphocytes was much slower than from nontolerant mice immunized in the same way, and it was close to the rate of elimination of the lymphocytes from intact mice. In the case of single immunization of the nontolerant mice with Streptococcus elimination of labeled lymphocytes only, and not of C. albicans, from the lymph nodes and spleen was intensified (compared with intact mice; Fig. 1). Immunization of the mice with E. coli vaccine did not change the rate of elimination of labeled C. albicans cells and C3H mouse lymphocytes from the tolerant or nontolerant mice.

These results show that tolerance to transplantation antigens of C3H mice produced in CC57BR mice in response to injection of C. albicans vaccine containing antigens related to transplantation antigens can be partially abolished by immunization with group A Streptococcus. In mice with partially abolished tolerance the length of survival of skin allografts and the rate of elimination of labeled allogeneic lymphocytes from the body were similar to the response observed in intact animals. The abolition of tolerance which the observed was connected with the specificity of the cross-reacting streptococcal antigens. Immunization with E. coli vaccine, not containing antigens related to tissue antigens, had no effect on the transplantation reactions in tolerant animals.

Investigation of serological reactions in tolerant mice after immunization with Streptococcus showed that tolerance to C. albicans antigens was not abolished (at least, to noncross-reacting antigens), for no immune response to the injected antigen was obtained during subsequent immunization with C. albicans. This was shown by the low rate of elimination of labeled C. albicans cells from the tolerant mice after their immunization with Streptococcus.

It can be concluded from the facts described above that immunization of mice tolerant toward C. albicans and related transplantation antigens with Streptococcus partially abolishes tolerance to transplantation antigens but not to antigens of C. albicans. However, the possibility cannot be ruled out that the abolition of tolerance to transplantation antigens observed after immunization with Streptococcus in the effect of summation of two coexisting states: 1) tolerance to transplantation antigens related to C. albicans and 2) sensitization arising to other alloantigens not related to C. albicans and cross-reacting with Streptococcus.

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