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Lymphocyte Reactivity in Genetic Tolerance

Kazuhiko Awaya

Emeritus Professor, Department of Anatomy, Yamaguchi University School of Medicine, Ube Yamaguchi 755, Japan
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Abstract A change in fast green histone staining of lymphocytes in skin allografted draining lymph node of F_1 hybrid (genetically tolerant) rats was detected by quantitative cytochemical examination. Such change was also found in allografted animal, but never in isografted and control (non-grafted) animals. These findings suggest that F_1 hybrid lymphocytes can recognize products of parental genes and react with them.

Key Words: Genetic tolerance, F_1 hybrid, Lymphocyte in tolerance, Fast green histone, Semi-allogenic graft

Introduction

The existence of "genetic tolerance" has been inferred largely on the basis of the universal failure of F_1 hybrid animals to reject grafts of parental strain skin. The validity of the inference has been challenged recently by demonstrations that F_1 hybrids may react against parental strain tissues. In the previous report we showed that the response of the regional lymph nodes of (DA × Lewis)F_1 hybrids to graft of DA skin resembled the lymph node response of allografted rats and was completely different from the response to the isograft[1]. The observation was performed at 3 days intervals after the skin graft. It was hoped to examine the lymphocyte reactivity of regional lymph nodes in earlier stages of skin graft. Histochemical change in nuclear histone content of lymphocytes were therefore examined as indication of a modification of the response attributable to immunological effects within 6 hours after skin semi-allograft.

Materials and Methods

Rats used in this experiment were from the inbred DA strain and (DA × Lewis)F_1 hybrids. Skin grafts of 4 cm^2 in area from the donor were placed on the right lateral thoracic wall of the recipients under sterile conditions. Three groups, each of 4 rats, were studied: (1) DA recipients of skin from DA donors (isograft group), (2) DA recipients of skin from (DA × Lewis)F_1 hybrid donors (allograft group) and (3) (DA × Lewis)F_1 hybrid rats grafted with skin from DA rats. Three and 6 hours after skin graft the rihgt superficial axillary lymph nodes draining the graft site were removed for examination. The rats were kept alive indefinitely following removal of lymph nodes in order to observe the subsequent course of the graft.

DA rats and (DA × Lewis)F_1 hybrids without having skin graft served as corresponding controls to DA rats and (DA × Lewis)F_1 hybrids bearing grafts.

Quantitative estimation of histone content of lymphocyte nuclei was performed as follows. Smears of lymphocytes from lymph nodes of the

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control group were made on one half of a cover glass (25 × 50 mm, No.1) and smears of lymphocytes from three experimental groups on the other half of the same cover glass. The smears were fixed for 10 minutes in 10% neutral buffered formalin. Hydrolysis with 5% trichloroacetic acid was performed at 90℃ for 15 minutes according to the method of Alfert and Geschwind[2]. The smears were stained in a 0.1% solution of fast green FCF (Chroma), buffered at pH 8.0 for 30 minutes at room temperature. Then, the smears were washed in distilled water, dried and mounted in immersion oil. The content of fast green-stained material in individual small lymphocytes was measured at 635 nm with microspecrophotometer (Olympus MSP-AIV). In each smear 30 cells were measured and a mean value calculated.

Results

As both the experimental and control smears were prepared on a single cover glass, they were stained under the same conditions throughout all procedures. Mean values of fast green histone in small lymphocytes of control rats were expressed as 100%. Data obtained are shown in Table 1.

A singnificant decrease in relative amount of fast green histone of small lymphocytes in the regional lymph nodes of the semi-allograft group (DA → F_1 skin graft transfer) was observed 3 hours after grafting. The transplanted skin graft was maintained more than 12 months for observation because (DA × Lewis) F_1 hybrid rats were "genetically tolerant" of DA skin. Similarly, a transient decrease in nuclear histone content of lymphocytes was found in the regional lymph nodes of the allograft group (F_1 → DA skin graft transfer). In this combination of donor and recipient, graft rejection occurred by the 12th day. Such a decrease of nuclear histone content in the lymph nodes was not seen in isograft group (DA → DA skin graft transfer). The grafted skin remained intact for longer than 12 months.

Table 1 Relative amount of fast green-histone in the small lymphocytes of the superficial axillary lymph nodes draining skin graft. Mean ± Standard errors

Group (Skin graft transfer)	3 hours	6 hours
Isograft (DA → DA)	102.4±5.5	95.2±2.9
Allograft (F_1→ DA)	88.0±3.2*	85.6±4.6*
Semi-Allograft (DA → F_1)	87.6±4.5*	94.1±3.3
Control (No graft)	100	100

*$p<0.05$
F_1 : (DA × Lewis) F_1
Control: Nontreated DA or F_1 hybrid
The values of control rats are expressed as 100%.

Discussion

The early reactivity of lymphocytes in regional lymph nodes draining the semi-allograft and allograft skin were examined. The changes in relative amount of fast green histone of lymphocyte nuclei were used as an indication of the early reactivity.

A significant but transient decrease of nuclear histone content of lymphocytes in the regional lymph nodes of semi-allograft recipient rats was observed at 3 hours after the skin graft. Such a lymphocyte reactivity also occured in the lymph node draining the skin allograft but not in that of isograft recipients. On the other hand the semi-allogeneic skin grafts transplanted with DA skin to (DA × Lewis) F_1 hybrid rats remained intact for longer than 12 months. Thus, in contrast to the fate of the graft which resembled that of skin isograft, the early reactivity of lymphocytes, i.e. the decrease in nuclear histone content, was very similar to that of allograft. This shows that the F_1 hybird lymphocytes may be able to recognize the parental DA strain skin graft as foreign.

There are some findings suggesting that in vitro experiments the histone molecules bound to the DNA can inhibit several enzyme systems resoponsible for DNA replication or for RNA transcription from DNA templates, and removal of histones bound to the DNA

enhances activity of the enzymes[3]. It is also well known that acetylation, methylation and phosphorylation of histones, which remove the positive charge, occur transiently at one point in the cell cycle[4]. Hence, it is logical to assume the decrease in relative amount of nuclear histone of small lymphocytes might be closely related to the early events in gene activation for DNA synthesis of lymphocytes in regional lymph nodes after the skin graft.

Further, it has been demonstrated that F_1 hybrids possess anti-idiotypic reactivity against the parental strain anti-F_1 determinant[5]. The lymphocyte reactivity of F_1 hybrid rats in the present study may reflect an early phase of this type of reactivity.

As reported previously, the response of the lymph nodes draining the semi-allografts was morphologically identical to that of the nodes draining allograft, the most important feature of this being the presence of large numbers of pyroninophilic cells in the medullary cords. Whether these histological features at a late stage following the skin graft are in direct continuity of the early reactivity of lymphocytes just mentioned is uncertain at the moment. This must await further substantiation.

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