Lymphocyte Disintegration and Plasma Cell Proliferation in Lymphatic Tissues: A Preliminary Report*

Kazuhiko AWAYA, Hiromu HORI and Masayoshi ODA
Department of Anatomy, Yamaguchi University, School of Medicine, Ube, Japan
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The most conspicuous feature in lymphatic tissues that occur after total-body X-irradiation is a massive destruction of lymphocytes followed by marked plasma cell proliferation (Wohlwill and Jetter 1953, Imamura 1959, Awaya et al. 1963). This fact indicates that the destruction of lymphatic tissue, and of lymphocytes in particular may cause the plasma cell proliferation. This train of idea suggests a search for similar reactions after transfer of degenerated lymphocytes. The present paper is a preliminary report on this subject.

MATERIAL AND METHODS

Young adult rats of a subline of Wistar strain, randombred and raised in the animal colonly of this laboratory, were used as material. The animals were divided into three groups and were intraperitoneally injected with (1) homogenate, (2) nuclear fraction and (3) cytoplasmic fraction of cells obtained from lymphoid organs of other animals of the same strain, respectively. For preparation of the nuclear and cytoplasmic fractions from the lymphoid cells the method of Sekiguchi and Shibatani of 1959 was employed. This is a modification of the method of Allfrey et al. of 1957. All the procedures were performed at 2°C. Thymus, mesenteric lymph nodes and spleen were rapidly removed from the sacrificed animals. The required tissues were finely minced with scissors and homogenized in a Potter-Elvehjem type glass homogenizer with 0.25 M sucrose-0.0055 M $Cacl_2$. The homogenate was replaced in a blendor vessel and gently homogenized by running the blendor at 1000 R.P.M. for 4 minutes. The resulting homogenate was filtered through a double layer of gauze and then through a single thickness of double napped flannelette. A portion of this filtrate was used as homogenate of lymphoid cells. The remaining filtrate was centrifuged at 2000 R.P.M. for 8 minutes and the supernate was used as cytoplasmic fraction. The sediment was resuspended in 0.25 M sucrose-0.002 M $Cacl_2$ and the suspension was again passed through flannelette. The filterate

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was centrifuged at 2000 R.P.M. for 8 minutes and the supernate was discarded. The sediment was resuspended in 0.25 M sucrose-0.002 M $Cacl_2$ and the suspension was used as nuclear fraction. An amount of homogenate, nuclei or cytoplasms equivalent to about 4000×10^6 cells was injected intraperitoneally into a rat of each group. Preliminary cell counts done before homogenation of lymphoid tissues showed that about 99 per cent of cellular element of suspension were lymphocytes of various sizes.

Sacrificing the animals 5 days after injection, mesenteric lymph nodes and spleen were excised and their imprint preparations were prepared. After drying and fixing, these preparations were stained with May-Giemsa. In the present study, plasma cell counts (%) from these preparations were mainly observed. The classification of cells was made by counting 2000 cells per organ in groups of 5 to 6 animals. In addition, the methyl green-pyronine stain was employed for identification of cells. As a control, to obtain base line figures, the mesenteric lymph nodes and spleen of 18 to 21 normal rats were examined in the same manner. We included in plasma cells not only cells of typical Marshalko type (mature form) but also what appeared to be those of relatively young stage. The latter were distinguished from the typical cells by their larger nuclei, but the size of the cytoplasm, the presence of a perinuclear clear zone and the absence of nucleolus were common to both types.

RESULTS AND DISCUSSION

The results of differential counts made on the mesenteric lymph nodes and spleen in normal and recipient rats are shown in Tables 1 and 2. In both organs of all the recipient groups, plasma cells were greatly increased in percentage as compared with those in normal rats, and in every group the increase in plasma cells was statistically significant ($P<0.05$ or $P<0.01$). It was further observed that plasma cell counts were higher in nats receiving the nuclear fraction than in those receiving the homogenate os cytoplasmic fraction of lymphoid tissues, although the difference between them was not significant ($P>0.05$).

These findings suggest that the products of lymphatic tissue disintegration may stimulate the plasma cell proliferation. We might attribute to a product of destroyed lymphocytes a role of stimulation of plasma cell proliferation, since we have observed that about 99% of cellular element in lymphoid tissues used for injection was lymphocytes of various sizes.

It must be taken into consideration, however, that since the Wistar rats employed in the present study were not inbred in the strictly genetic sense the lymphoid tissues used for injection might posess antigenicities for recipients, so that plasma cells might proliferate as a results of immune response in the host. Furthermore, it should be added here that lymph node homograft in vivo in a

Table 1. Differential cell counts in the mesenteric lymph nodes of normal rats and of those injected intraperitoneally with homogenate, cytoplasmic and nuclear fraction of lymphoid cells obtained from the thymo-lymphatic tissues of other animals of the same strain and killed after 5 days.

Group	Animal No.	Plasma cells		Lymphocytes				Granulocytes	Reticulum cells	Others
		Mean ± S. E.	Range	Large	Medium-sized	Small	Total			
Normal	18	3.7±0.90	0.1−10.0	0.2±0.07	3.9±0.33	91.0±0.56	95.1±1.02	0.4±0.10	0.7±0.10	0.1±0.05
Homogenate-injected	5	8.4±0.53*	7.2−10.3	0.6±0.20	9.2±1.01	80.4±1.85	90.2±1.02	0.5±0.09	0.8±0.30	0.1±0.05
Cytoplasmic fraction-injected	6	8.3±1.66*	3.3−14.0	0.1±0.04	2.9±0.47	84.4±3.25	87.4±3.16	1.0±0.33	2.2±1.12	1.1±0.39
Nuclear fraction-injected	6	17.3±7.94**	6.0−56.0	0.2±0.06	4.4±0.64	76.0±8.58	80.6±8.07	0.4±0.37	1.3±0.29	0.4±0.19

* These values are significant at the level of $P<0.05$ compared with normal value.

** These values are significant at the level of $P<0.01$ compared with normal value.

Table 2. Differential cell counts in the spleen of normal rats and of those injected intraperitoneally with homogenate, cytoplasmic and nuclear fraction of lymphoid cells obtained from the thymo-lymphatic tissues of other animals of the same strain and killed after 5 days.

Group	Animal No.	Plasma cells		Lymphocytes				Granulocytes	Reticulum cells	Others
		Mean ± S. E.	Range	Large	Medium-sized	Small	Total			
Normal	21	0.7±0.11	0.1−1.5	0.7±0.09	3.9±0.46	84.5±1.20	89.1±1.06	2.9±0.34	0.4±0.03	6.9±0.64
Homogenate-injected	5	5.1±0.69*	3.1−6.5	0.1±0.06	11.2±1.66	66.3±2.49	77.6±2.04	9.1±1.98	3.0±0.71	5.2±1.16
Cytoplasmic fraction-injected	6	4.6±0.39*	3.5−6.0	0.1±0.03	10.2±0.58	75.4±1.64	85.7±1.36	5.1±1.24	2.5±0.46	2.1±0.48
Nuclear fraction-injected	6	5.8±0.56*	3.5−6.8	0.1±0.06	11.0±1.11	71.9±3.37	83.0±3.15	5.5±2.16	2.1±0.42	3.6±1.35

* These values are significant at the level of $P<0.01$ compared with normal value.

diffusion chamber induces a plasma cell proliferation in host tissues, particularly in those surrounding the chamber during the breakdown of lymph node homograft (AWAYA, 1962). In order to exclude such a possible fashion of plasma cell proliferation, a series of experiments with inbred mouse along the same line as reported in the present paper are now in progress.

SUMMARY

The intraperitoneal injection of homogenate, nuclear or cytoplasmic fractions of lymphoid cells into other rats of the same strain causes a conspicuous plasma cell proliferation in host lymphatic tissues. This suggests a possibility that a product of destroyed lymphocytes might promote the plasma-cellular reaction.

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