Histopathology of Regression of Tumor Metastasis in the Lymph Nodes

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SUMMARY

A transplanted rat lymphosarcoma (WGT-4), induced by Gross virus and inoculated s.c. into the foot of a normal syngeneic rat, initially grew but ultimately regressed. The tumor cells metastasized to the regional popliteal, lumbar, and inguinal lymph nodes and formed massive metastatic foci there. These lymph node metastases also regressed spontaneously. However, in Gross-tolerant rats inoculated with Gross virus at birth, no regression was observed. Histopathologically, infiltration and proliferation of lymphoid cells, reticulum cells, and fibrocytes occurred in the regressing metastatic tumor in lymph nodes as well as in the regressing transplanted tumor in the foot. Only in lymph nodes of normal rats, in which tumor metastasis regressed, was the characteristic "starry sky" appearance observed. Our results suggest that regression of metastatic tumor in lymph nodes, as well as of transplanted tumor in syngeneic rats, was due to an immunological reaction by the host and that an immunological factor may be responsible for the "starry sky" picture.

INTRODUCTION

The morphology and biology of experimental tumor metastasis in lymph nodes, especially its occurrence and development, have been extensively studied (5, 11, 18, 19). However, only a few reports have dealt with the regression of metastatic tumors in lymph nodes in syngeneic tumor-bearing hosts (10, 20). Kobayashi et al. (7, 8) showed that Friend, Rauscher, and Gross virus-induced tumors that developed in rats given injections of each of these viruses at birth grew only in the Friend, Rauscher, and Gross virus-conditioned and -tolerant rats and that they did not grow in syngeneic normal adult rats. One line of such rat tumors, induced by Gross virus (rat Gross tumors) and transplanted s.c., showed temporary growth at the inoculation site in syngeneic rats forming metastasis in draining lymph nodes. The tumor eventually regressed, with accompanying spontaneous regression of lymph node metastasis. These studies evaluate the histopathology of rat Gross tumor transplanted s.c. and of metastasis to draining lymph nodes, with special reference to the immunological regression of tumor metastasis in the lymph nodes.

MATERIALS AND METHODS

Tumor and Rats. WGT-4 tumor, one line of the rat Gross tumors, was established from the thymus of the WKA/Mk rat 106 days after the neonatal injection of Gross virus. Biological characteristics of this tumor in either solid or ascites form have been detailed previously (8). Briefly, when WGT-4 tumor was transplanted either s.c. or i.p. into normal adult WKA/Mk rats, the tumor grew temporarily as a solid or ascites tumor but later regressed. In order to maintain the tumor, WKA/Mk rats that had been given injections of Gross virus at birth, referred to as Gross-tolerant rats, were used. The mean survival after transplantation of the tumor was 15 days. Histocompatible WKA/Mk rats were used for experiments. They were bred in the Experimental Animal Center, Faculty of Science, Hokkaido University, Sapporo, Japan. Gross-tolerant rats, 2-month-old WKA/Mk rats that had been given s.c. and i.p. injection of undiluted Gross virus within 48 hr after birth, were also used. These rats were viremic and tolerant to Gross virus-induced transplantation antigen as reported previously (8, 12).

Procedures. Thirty-four Gross-tolerant and 34 normal rats weighing 90 to 120 g each, received a single injection of $5 \times 10^6$ living tumor cells in the right foot. At 2, 4, 6, 8, 10, 13, and 16 days after injection 4 Gross-tolerant and 4 normal rats were killed for examination, while the 6 remaining were observed until death from tumor growth.

Animals were killed with ether and were autopsied. Tumor size in the right foot and right popliteal, lumbar, and inguinal lymph nodes draining the tumor site was measured and the mean diameter was recorded. Portions of tissue were fixed in 4% formaldehyde solution, sectioned, and stained with hematoxylin and eosin, Azan Mallory's stain for collagen fibers, and Gomori's silver impregnation for reticulum fibers. In preparation for electron microscopic examination, tissues were fixed in 1% OsO$_4$ solutions. Sections were stained with uranyl acetate and lead hydroxide.

RESULTS

Growth of Transplanted Tumor. WGT-4 tumor inoculated into the right foot of Gross-tolerant WKA/Mk rats...
grew well, and all 6 rats died from tumor growth after approximately 16 days. However, tumor inoculated into normal rats showed temporary growth (maximum size of 10 mm at the 7th day) followed by regression. At 16 days on the average, all the WGT-4 tumors completely regressed. No recurrence of the tumor was observed for 6 months after regression. The average growth curve of WGT-4 tumor in Gross-tolerant and normal rats is given in Chart 1.

**Histology of Transplanted Tumor.** Histological examination of WGT-4 tumor grown in the right foot of Gross-tolerant WKA/Mk rats showed massive proliferation of uniform lymphoblastic cells. The tumor cells possessed a small amount of faintly basophilic cytoplasm and a large round nucleus occupying a central position with a rather indistinct nuclear membrane. When rats died from tumor, focal necrosis of the tumor due to ischemia was observed. WGT-4 tumor showed the same findings in normal rats as in Gross-tolerant rats by the 4th day after tumor inoculation (Figs. 1 and 2). After 6 days, however, abundant lymphocytes, reticulum cells, and fibroblasts appeared within the tumor. Degeneration, necrosis, and elimination of tumor cells occurred (Fig. 3). After 10 days these areas showed residual nests of fibroblasts containing plasma cells and lymphoid cells, but no tumor cells (Fig. 4).

**Enlargement of Lymph Nodes.** Right popliteal, lumbar, and inguinal lymph nodes draining the WGT-4 tumor site in Gross-tolerant WKA/Mk rats enlarged progressively with the tumor. At the time of death from tumor, lymph nodes were at their maximum size. Lymph nodes in normal rats inoculated with tumor enlarged temporarily, then returned to normal size. By the 8th day lymph nodes in rats inoculated with WGT-4 tumor reached a maximum size of 9 mm, 4 times their size in the normal control rats, and then returned to normal size at approximately 16 days. The average size of the right lumbar lymph node in Gross-tolerant and normal rats after inoculation with tumor is presented in Chart 2.

**Histology of Tumor Metastasis in Lymph Nodes.** Lymph node metastasis of WGT-4 tumor inoculated into Gross-tolerant and normal WKA/Mk rats first appeared in some animals at Day 2. The area of invasion of the tumor cells was from the marginal sinus to the intermediary sinus rather than the cortical pulp. On Day 4 all lymph nodes that were examined, i.e., right popliteal, lumbar, and inguinal nodes, showed tumor metastasis of various grades. In some cases lymph nodes were fully occupied by tumor cells without any normal structure (Figs. 5 and 6). The presence and the grade of metastasis in lymph nodes in Gross-tolerant and normal rats are shown in Table 1. Lymph node metastases were graded according to the 3 classifications of Kurokawa-Sato, as previously detailed (11). Briefly, Grade I represents the proliferation of metastatic tumor cells in the marginal sinus. In Grade II tumor cells proliferate as far as intermediary sinus and invade into parenchyma. The tumor cells occupy almost all the lymph node in Grade III. In Gross-tolerant rats inoculated with WGT-4 and killed on Day 16, lymph node metastases were observed in the highest frequency and grade. However, in all metastatic lymph nodes of normal rats, characteristic "starry sky" appearance, similar to that seen in Burkitt's lymphoma, with a uniform background of tumor cells studded with large phagocytic histiocytes, was recognized on Day 6 (Figs. 7 and 8). Histiocytes containing pyknotic cell debris were prominent in metastatic lymph nodes on Day 8 (Figs. 9 and 10). WGT-4 tumor cells in these lymph nodes rapidly decreased indicating disappearance of metastatic foci. Tumor cells with signs of degeneration occurred only cortical areas accompanied by infiltration of reticulohistiocytes, fibrocytes, and lymphocytes (Figs. 11 and 12). Ten days after inoculation, no tumor cells were observed in all lymph nodes examined.

Histological changes of elements in lymph nodes with tumor metastases such as follicles, sinus histiocytes, large mononuclear cells, and plasma cells were examined. The usual nomenclature of these lymphoid cells was followed (1, 3, 5). The histiocyte was irregularly shaped with abundant cytoplasm and ovoid nucleus containing finely clumped chromatin. The large mononuclear cell has basophilic cytoplasm and a large nucleus with 1 or 2 prominent nucleoli. A summary of the results obtained from findings
of lymph nodes at 2 days (early stage), 6 days (middle stage), and 10 and 13 days (late stage) is given in Table 2. In the lymph nodes of Gross-tolerant rats, progressive proliferation of metastatic tumor cells, no regression of tumor growth, increase of large mononuclear cells and plasma cells were observed only during the middle stage. After the 10th day following tumor inoculation, almost all of the lymph nodes were occupied with tumor metastasis and lymph node elements had disappeared. On the other hand, marked changes of lymph node elements were observed in normal rats in which tumor growth and lymph node metastasis eventually regressed. Temporal disappearance of follicles, marked sinus histiocytosis, and prominent increase of both large mononuclear cells and plasma cells were seen in these lymph nodes (Figs. 13 to 18).

_Ultrastructural Findings._ Six days after inoculation of WGT-4 tumor cells, right lumbar lymph nodes in normal and Gross-tolerant rats were examined by electron microscopy. A low-power micrograph of round or oval WGT-4 tumor cells in Gross-tolerant rats, demonstrating the thin cytoplasm and the large oval nucleus with several indentations and prominent nucleoli, appears as Fig. 19. Virus particles were found in intercellular spaces. The cytoplasmic composition was like that of lymphoblast (Fig. 20). It contained many free ribosomes, a few channels of endoplasmic reticulum, large mitochondria with pale internal material, and Golgi vesicles. In lymph nodes of normal rats, which histologically showed a “starry sky” appearance, many histiocytes surrounded with tumor cells were found. As shown in Fig. 21, the histiocyte had an eccentric nucleus with the heterochromatin generally evenly dispersed. One or 2 nucleoli were usually present. The cytoplasm contained a large number of vacuoles, lysosomes, and large phagosomes. However, there was no morphological finding that these histiocytes played a role in the rejection of the tumor cell.

**DISCUSSION**

Friend, Gross, and Rauscher virus-induced tumors in rats have been grown only in Friend, Gross, and Rauscher-tolerant or immunosuppressed rats (8, 9) and not in normal adult rats (7, 8). Spontaneously developed or chemically induced tumors have not grown in syngeneic normal rats after they were artificially infected with the Friend, Gross, and Rauscher viruses (8, 9). The above findings are similar in nature to, and may be referred to as, “xenogenization of tumors,” which is defined as the immunological regression of tumors without previous immunization in the host. Xenogenization of tumors has been studied by transplantation, immunofluorescence, and cytotoxicity tests (8, 9, 12).

In this experiment, Gross virus-induced WGT-4 lymphosarcoma, inoculated into the foot of normal syngeneic rats,
initially grew but eventually regressed. In Gross-tolerant rats, however, WGT-4 tumor grew well and killed the host. These results indicate that the regression of WGT-4 tumor in normal syngeneic rats occurred because of the immunological rejection in the host by a mechanism similar to xenogenization of tumor.

Histological examination of WGT-4 tumor at the site of inoculation in normal rats revealed that the tumor tissues were composed of proliferative and infiltrative tumor cells by the 4th day after inoculation. In the tumor tissues of normal rats after the 6th day, degeneration, necrosis, and disappearance of the tumor cells occurred, accompanied by reactive infiltrations of lymphocytes, reticulum cells, histiocytes, and fibrocytes. At 10 days the tumor cells disappeared completely from the site of inoculation. These findings are similar to the histology of homograft rejection of normal skin, kidney, tumor, and autochthonous tumors induced by murine sarcoma virus (14-16). Similar findings were also obtained in a regressing tumor after artificial infection of a normal syngeneic rat with Friend virus, as previously reported (10). Therefore, it is strongly suggested from the histological characteristics of our study that the regression of WGT-4 tumor in normal rats was due to immunological reaction by the host.

One characteristic result of this study was that WGT-4 tumor cells metastasizing to lymph nodes regressed spontaneously in normal rats. In experimental reports on tumor metastases in lymph nodes, many investigators described the mechanism of formation and development of metastasis. Only our report has dealt with the spontaneous regression of tumor metastasis developed in the lymph nodes of a syngeneic tumor-bearing host without the aid of previous immunization in the host (10). Histology of regression in lymph node metastasis of the tumor accompanied by reactive infiltration of reticulum cells and histiocytes was also similar to that seen in the WGT-4 tumor tissue regressing in the foot of normal rats. Morphological changes in the lymph nodes with WGT-4 tumor metastasis were the disappearance of lymph follicles and the proliferation of large mononuclear cells, plasma cells, and sinus histiocytes. These were noted in the lymph nodes of the host given antigenic stimulation; we also recognized these changes in the lymph nodes with regressing metastatic tumor in rats artificially infected with Friend virus (1, 3, 4, 10). Appearance of a large number of plasma cells and large mononuclear cells suggests that a humoral antibody mechanism might also play a role in the rejection of the tumor, in addition to cell-mediated immunity.

The most characteristic finding emerging from this work was the appearance of "starry sky" produced by a uniform background of WGT-4 tumor cells studded with large phagocytic histiocytes in the regressing metastatic tumor in lymph nodes. The appearance of "starry sky" was originally observed in Burkitt's lymphoma. It has been suggested that an immunological factor might be responsible for its appearance (2, 6, 13). In our examination, "starry sky" was found only in the lymph nodes of normal rats in which metastasis of WGT-4 lymphosarcoma regressed. The histological picture of clear histiocytes in metastatic lymph nodes, which suggested the changing from "starry sky" appearance, was found in the late stage of the regression in lymph node metastasis of WGT-4 tumor. Therefore, it was suggested that certain immunological factors are related to this appearance. However, at present we have no evidence, histologically or electromicroscopically, that these histiocytes play a role in the rejection of the tumor cells. Further study of this appearance will be made with special reference to significant morphology of the immunological phenomenon.

REFERENCES

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Figs. 1 to 18 were stained with H & E.
Fig. 1. WGT-4 tumor in foot of normal rats 4 days after inoculation. × 250.
Fig. 2. WGT-4 tumor, lymphosarcoma cells in foot 4 days after inoculation. × 400.
Fig. 3. WGT-4 tumor in foot of a normal rat 6 days after inoculation. Arrows, degenerative changes of tumor cells. Infiltration of reticulohistiocytes and fibroblasts are shown. × 400.
Fig. 4. Tissue in foot of normal rat 10 days after inoculation of WGT-4 tumor. Infiltration of fibroblasts accompanied by lymphoid cells is shown. × 300.
Fig. 5. Lymph node metastasis of WGT-4 tumor in normal rat 4 days after inoculation. Tumor cells deeply infiltrate cortical and medullary areas. × 150.
Fig. 6. Metastasis of WGT-4 tumor cells that invaded medullary area 4 days after inoculation into normal rats. × 300.
Fig. 7. "Starry sky" appearance of metastatic foci of WGT-4 tumor in lymph nodes of normal rats 6 days after inoculation. × 100.
Fig. 8. Higher magnification of "starry sky" appearance. Arrows, clear histiocytes laden with pyknotic cells or cell debris. × 400.
Fig. 9. Sparse appearances of metastasis of WGT-4 tumor cells in lymph node 8 days after inoculation into normal rat. × 100.
Fig. 10. Higher magnification of reticulohistiocytic reactions intermixed with lymphoid cells and clear histiocytes in WGT-4 tumor metastasis in lymph nodes of normal rats 8 days after inoculation. × 450.
Fig. 11. Granulomatous pattern of metastatic foci of WGT-4 tumor in lymph node of normal rat 8 days after inoculation. × 100.
Fig. 12. Degenerating WGT-4 tumor cells (arrows) in cortical area of lymph node in normal rat 8 days after inoculation. × 400.
Fig. 13. Disappearance of follicles in lumbar lymph node of normal rat 2 days after inoculation of WGT-4 tumor. × 80.
Fig. 14. Reappearance of follicles in lumbar lymph node of normal rat 13 days after inoculation of WGT-4 tumor. × 100.
Fig. 15. Sinus histiocytosis in lumbar lymph node of normal rat 10 days after inoculation of WGT-4 tumor. × 80.
Fig. 16. Higher magnification of sinus histiocytosis in lymph node of normal rat 10 days after inoculation of WGT-4 tumor. × 400.
Fig. 17. Proliferation of large mononuclear cells in medullary area of WGT-4 tumor-metastasized lymph node of normal rat 6 days after inoculation. × 450.
Fig. 18. Plasmocytosis in cortical pulp of WGT-4 tumor-metastasized lymph node of normal rat 6 days after inoculation. × 450.
Figs. 19 to 21 were stained with uranyl acetate and lead hydroxide.
Fig. 19. WGT-4 tumor cells in lumbar lymph node of Gross-tolerant rat 6 days after tumor inoculation. Lymphoblastic-like tumor cells are shown. × 2500.
Fig. 20. Tumor cell containing many free ribosomes, a few channels of endoplasmic reticulum, large mitochondria, and Golgi vesicles. Arrow, virus-like particles in intercellular spaces. × 8000.
Fig. 21. Histiocyte surrounded with tumor cells. A large number of vacuoles, lysosomes, and large phagosomes are shown. No interaction between histiocyte and tumor cells are shown morphologically. × 4000.
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