CYTOLOGICAL EVIDENCE FOR A RELATIONSHIP BETWEEN NORMAL HEMATOPOIETIC COLONY-FORMING CELLS AND CELLS OF THE LYMPHOID SYSTEM*

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Plates 61 and 62

(Received for publication 9 October 1967)

We have obtained cytological evidence that mouse hematopoietic colony-forming stem cells can differentiate along both erythropoietic and granulocytic lines (1). This class of stem cells is present in normal bone marrow, a tissue known from the experiments of Ford et al. (2) and Micklem et al. (3) to contain cells capable of repopulating not only the myeloid tissues but also the lymphoid tissues of irradiated mice. While it seems probable that the colony-forming cells of marrow might be responsible for repopulating both tissues (4, 5), conclusive proof for their differentiation to lymphoid cells is not available. In the present paper we present evidence showing that single clones, whose members are identified by the presence of unique chromosomal markers, may contain thymus cells and cells of lymph nodes in addition to hematopoietic colony-forming cells. These findings mean either that lymphoid cells are descended from colony-forming cells or that both classes have a common progenitor.

Materials and Methods

Mice.—The mice used in these experiments were F_1 hybrids between mice of genotype WB-W/+ and C57 BL/6-+/W^v. This cross yields normal F_1 hybrids (genotype WBB6-+/+), mice heterozygous at the W locus (genotypes WBB6-W/+ and WBB6-+/W^v), and mice of genotype WBB6-W/W^v (6).

Generation of Hematopoietic Cells with Abnormal Karyotypes.—The two-stage technique used for generating hematopoietic cells with characteristic chromosomal markers was the same as that described previously (1). It is based on the use of gamma rays to induce chromosomal markers in individual hematopoietic stem cells; irradiated suspensions containing such

* This work was supported by the Medical Research Council (Grant MBT-1420), the National Cancer Institute of Canada, the Jane Coffin Childs Memorial Fund for Medical Research, and the Defence Research Board (Grant 9350-14 G & C).

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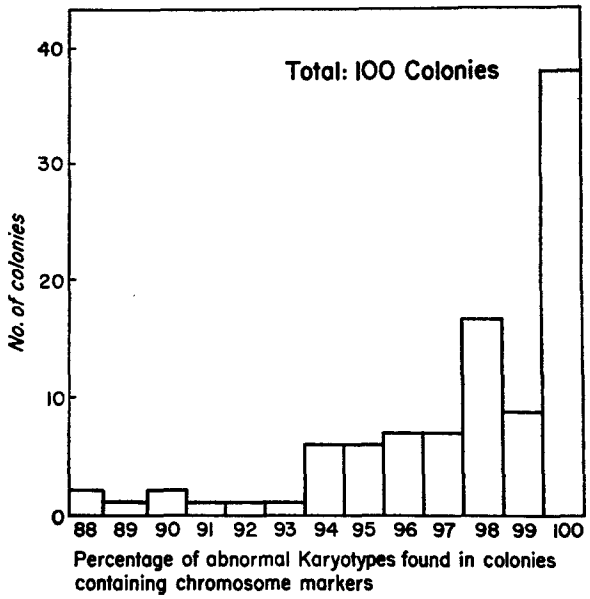
cells are injected at or near limiting dilution into unirradiated genetically anemic mice of genotype W/W^v. These recipients have defective stem cells unable to form macroscopic spleen colonies; they are, however, able to support the growth and differentiation of normal stem cells obtained from coisogenic littermates (7). The presence of normal stem cells growing in mice of genotype W/W^v can be detected by their capacity to form spleen colonies; in addition the tissues of W/W^v mice may be repopulated from a marrow graft too small to permit the survival of heavily irradiated mice. The procedure was as follows: Animals of normal genotype (WBB6-+/+) received a large dose (usually 850 rads) of gamma radiation. Marrow suspensions containing 2×10^7 cells from these animals were injected into each of a



TEXT-FIG. 1. Distribution of the frequency of metaphase cells having a characteristic chromosome marker among 100 spleen colonies in which abnormal chromosomes were found.

group of mice of genotype WBB6-W/W^v. After a period of approximately 2 months, cells from the marrows or thymuses of these recipient animals were examined for the presence of cells with abnormal karyotypes. If these were found, the marrow was transplanted into heavily irradiated recipient mice to produce spleen colonies. These recipients were either of normal genotype or heterozygous at the W locus, and were given 950 rads of total-body irradiation prior to marrow transplantation.

It was shown previously that the two-stage technique described above is an efficient method for obtaining marrow-derived spleen colonies, each with a characteristic chromosomal marker present in high frequency (1). This result was confirmed in the present studies. Text-fig. 1 shows the results of an analysis of 100 such marked colonies. It is evident from the figure that, in approximately 40% of the colonies, the characteristic marker was present in 100% of the metaphases examined. In the remainder of the colonies, a small percentage of unmarked contaminating cells was present, as reported earlier (1).

Chromosomal Analysis.—Chromosomal analysis of mitotic cells from individual colonies

and from the tissues of the recipient animals was carried out on preparations obtained using a modification of the technique of Bunker (8) as described previously (9).

Examination of Lymph Nodes.—Lymph nodes were examined after antigenic stimulation. This was accomplished by injecting 0.1 ml of washed packed sheep erythrocytes into one foot-pad daily for 4 consecutive days. On the 5th day, the nodes were either removed surgically under light pentobarbitone anesthesia or at the time of sacrifice of the animals. Cell suspensions from the nodes were examined cytologically for the presence of abnormal chromosomes. In addition, the cell suspensions were tested for hemolysin-producing cells by the technique of Jerne and Nordin (10) as modified by Kennedy et al. (11).

RESULTS

Relationship between Hematopoietic Spleen Colony-Forming Cells and Cells of the Thymus.—In the experiments to be reported below, the use of radiation-induced chromosomal markers to identify members of a clone of cells was combined with the use of the spleen colony technique to recognize hematopoietic stem cells (see Materials and Methods). This combination of methods permits clones of cells related to hematopoietic stem cells to be identified regardless of of their location in the mouse. To relate thymus cells with hematopoietic stem cells the following procedure was carried out: Mice of genotype W/W^v received injections of marrow from heavily irradiated donors as described in Materials and Methods. After 2–6 months, the animals were killed and their thymus glands were examined for the presence of chromosomal markers. A high percentage of chromosomal markers was found in the thymus glands of 8 animals out of 51 examined. Among the remainder of the animals, 15 showed marked cells only in the marrow, six had infrequent marked cells in the thymus as well as the marrow, and the rest were free of marked cells.

Marrow from six of the eight animals that had shown a high percentage of marked cells in the thymus was injected into heavily irradiated coisogenic normal or heterozygous mice, and the resulting spleen colonies were examined after 14 days for the presence of cells with abnormal karyotypes.

Table I gives the findings from the eight animals selected because of a high percentage of marked cells in the thymus (see column 6 of Table I). The same chromosomal markers were always found in the marrow of these animals, although with variable frequency (Table I, column 7). When spleen colonies derived from this marrow were examined, some colonies, having the characteristic marker in all or almost all of their dividing cells, were always found (Table I, column 8). However, the proportion of such marked colonies was variable and tended to be correlated with the frequency of the marker in the marrow.

The analyses of three of the mice (Table I) deserve special comment. Mouse 5 contained two different markers. One of these, a metacentric, is shown in Fig. 1. It was present in 80% of the dividing cells of the thymus. This marker, however, was present in only 12% of the cells of the marrow and 3 out of 15 of the spleen colonies examined. The marrow of this same animal contained cells bear-

ing another pair of characteristic chromosomal markers, a long chromosome and a minute chromosome. These markers were not present in the thymus, but were present in 48% of the marrow metaphases examined and 12 out of 15 of the spleen colonies derived from this marrow. Mice 2 and 8 are also of interest. In these animals approximately 50% of the cells in the marrow contained characteristic markers; in both cases the markers present in the cells of the marrow were

TABLE I

Distribution of Cells with Abnormal Karyotypes among Thymus, Lymph Nodes, Bone Marrow, and Bone Marrow–Derived Spleen Colonies

Exp. No.	Mouse No.	Time interval*	Abnormal chromosomes	Percentage of cells with marker‡			Spleen colonies§
				Lymph node‖	Thymus	Marrow	
		days					
I	1	65	Long	—	56	8	2/25
	2	65	2 minutes	—	80	48	24/24
II	3	84	Abnormal constriction and minute	0	88	4	2/14
	4	83	Long	0	76	48	13/14
	5	83	(*a*) Metacentric	0	80	12	3/15
			(*b*) Long and minute	0	0	48	12/15
III	6	78	Long	0	84	40	—
	7	86	Minute	0	88	32	—
IV	8	174	Metacentric and minute	65	84	52	7/7

* Time interval between marrow injection and sacrifice of the animal.

‡ 25 well-spread metaphase cells were examined in each case.

§ No. of colonies containing the characteristic marker/No. of colonies examined.

‖ Lymph nodes were removed surgically after antigenic stimulation, 18–25 days before animals were killed. Animal 8 was not given antigen and all of the organs were examined at the same time.

found in all of the spleen colonies derived from that marrow. Only seven colonies from the marrow of mouse 8 were examined; however, the marker was present in all of 100 metaphase cells obtained from a pool made from approximately 50 colonies derived from this marrow. In these two instances, therefore, all of the spleen colony–forming cells may be considered to belong to the same clone and, in respect to normal hematopoietic colony–forming cells and their progeny, these animals may be considered to be clonally repopulated.

The results of these investigations provide cytological evidence that a high proportion of the cells of the thymuses of mice of genotype W/W^v can belong to

the same clone as normal hematopoietic colony-forming cells, cells known to be capable of giving rise to erythropoietic and granulopoietic descendants (1).

Relationship of Cells of Lymph Nodes to Normal Hematopoietic Colony-Forming Cells.—Harris and Ford (13) have presented cytological evidence that cells present in thymic grafts migrate to the lymph nodes. Therefore, we examined lymph nodes from unirradiated W/W^v chimeras whose thymuses contained marked cells. In most instances, prior to examination, the mice were stimulated with antigen (see Materials and Methods). The results obtained from six animals are shown in column 5 of Table I. In five of these cases no marked cells were found in the lymph nodes, although metaphase cells for examination were found with ease. Mouse 8, however, was unique. Not only was this animal clonal in respect to the normal colony-forming cells found in its marrow, but also a high proportion of the metaphases in spleen, thymus, and lymph node contained the metacentric and minute markers characteristic of this clone (Fig. 2). Indeed, the markers were detected in 65% of the lymph node cells even though antigenic stimulation was not employed in this animal.

Mouse 8 of Table I differed from the others in that almost 6 months, rather than 2–3 months, had elapsed between the time of marrow transplantation and the time of cytological examination. It seemed possible, therefore, that this prolonged time was required for dispersion of the clone throughout the tissues of the mouse. However, in the experiments of Micklem et al. (3) repopulation of lymphatic nodes in irradiated mice occurred after only 3–6 wk. The results observed in our experiments might differ from those of Micklem et al. because of a reduction in cellular traffic between thymus and lymph nodes in unirradiated animals compared with irradiated mice. To test this possibility, an experiment in which cells bearing radiation-induced chromosomal markers were injected into irradiated recipients was performed as follows: A group of W/W^v mice was examined 4 months after the transplantation of marrow from irradiated recipients. An animal was selected in which 40% of the bone marrow metaphase cells contained a characteristic marker of the long and minute type. These marrow cells were used to generate spleen colonies in irradiated mice of normal or heterozygous genotype. A cell suspension was prepared from a pool of a large number of such colonies; all of 50 cells from this suspension were found to have the characteristic long and minute chromosomal markers. Aliquots containing 2×10^7 cells from this suspension were injected into heavily irradiated normal-mice. A month later, these recipients were stimulated with antigen (see Materials and Methods), their lymph nodes excised and examined both for the presence of chromosomal markers and antibody-producing cells. The results are presented in Table II. In all five animals the markers characteristic of this clone were found in some of the lymph node cells, and in two mice more than 65% of the metaphases examined contained the marker. In addition, these lymph nodes contained approximately 5–10 times as many antibody-producing cells, as

detected by the plaque-formation technique of Jerne and Nordin (10), as are found in normal mice. These findings indicate that cells belonging to the same clone as colony-forming stem cells may reach the lymph nodes of irradiated mice within 1 month, and that nodes containing cells of this origin can participate in an immunological response.

DISCUSSION

In the experiments reported in this paper, radiation-induced chromosomal markers were used to identify the progeny of single hematopoietic stem cells after extensive proliferation following transplantation into mice of genotype

TABLE II

Presence of a Characteristic Marker in the Lymph Nodes of Irradiated Mice given Transplants of Pooled Marked Cells from Spleen Colonies belonging to a Single Clone

Mouse No.	Marked cells/Total cells*	P.F.C. per 10^6 cells‡
1	67/100	502
2	5/8	238
3	3/7	200
4	2/9	119
5	9/13	357

* Number of marked metaphase cells/Total number of metaphase cells examined. The markers characteristic of this clone were a long chromosome and a minute chromosome.

‡ Lymph nodes were taken from mice immunized with sheep red cells as described in Materials and Methods. Cells from the lymph nodes of nonimmunized animals contain about 40 plaque-forming cells (P.F.C.) per 10^6 nucleated cells.

W/W^v. The use of marker chromosomes to examine the relationship between hematopoietic and lymphoid cells was introduced by Ford and his colleagues at Harwell (12); these workers have studied both chromosomally marked cells arising from the surviving stem cells of heavily irradiated mice (14), and bone marrow cells obtained from CBA-T6 mice (all of the cells of these mice have a characteristic chromosomal marker) transplanted into irradiated recipient coisogenic mice (2, 3). Our procedure differs from that of the Harwell group principally in the use of mice of genotype W/W^v as recipient animals. As a result of mutation at the W locus, these animals have defective hematopoietic stem cells that are unable to form macroscopic colonies following transplantation into heavily irradiated mice (7). However, normal colony-forming cells derived from the coisogenic littermates of mice of genotype W/W^v proliferate following transplantation into the genetically anemic animals and can subsequently be identified by their capacity to give rise to macroscopic spleen colonies. Irradiation of donor marrow prior to its transplantation into mice of genotype W/W^v results in the induction of unique chromosomal markers that

permit the subsequent identification of members of single clones even after these cells are widely disseminated in the tissues of the host.

Our results confirm those of the Harwell group in showing that cells present in the marrow have capacity to proliferate not only in marrow but also in thymus and lympatic nodes. The pattern of repopulation, however, was different in our unirradiated mice from that observed by Ford and his colleagues (2, 3). In their irradiated recipients, both thymus and lymphatic tissue were repopulated within approximately 1 month after marrow transplantation, and they have suggested that there is a cellular traffic occurring from marrow to thymus to lymph nodes in mice. Experiments on animals joined in parabiosis indicated that similar traffic exists in normal animals (15). While we found marked cells in marrow and thymus 2 months after marrow transplantation into unirradiated mice of genotype W/W^v, chromosomally marked cells were not identified in lymph nodes until almost 6 months had passed. Indeed, we failed to find cells in lymph nodes even at times when more than 50% of the dividing cells in the thymus contained characteristic markers. We interpret this finding as indicating that cellular migration from thymus to lymph nodes occurs much more slowly in unirradiated animals than in irradiated mice, and that, therefore, this cellular traffic may be more significant during recovery from injury than under normal physiological conditions.

Our experimental procedures allowed us to relate cells found in thymus and lymph nodes to normal hematopoietic colony-forming cells, a class of stem cells usually found most plentifully in marrow and spleen of mice. These findings, taken with those of a previous study (1), indicate that hematopoietic colony-forming cells, erythroblasts, granulocytes, thymic cells, and the cells of lymph nodes may all belong to the same clone. Our studies, however, do not allow us to determine precise parent-progeny relationships within such a clone. For example, we do not know whether or not some or all hematopoietic colony-forming cells can differentiate to give rise to both myeloid and lymphoid descendants or, alternatively, if colony-forming stem cells and lymphoid cells have a common, as yet unidentified, precursor. The resolution of this problem will require further detailed analysis of patterns of differentiation within large hematopoietic clones.

Our studies of lymphoid tissue showed that cells containing the same chromosomal markers as hematopoietic colony-forming cells could be present in more than 50% of the dividing cells during an immunological response. The present evidence, taken together with the recent results of Trentin and coworkers (16) strongly indicates that antibody-producing cells and hematopoietic colony-forming cells may belong to the same clone. However, as yet, it has not been shown that cells with the characteristic marker are, in fact, producing antibody. If this final requirement could be met, then the techniques described in this paper should provide a means for producing large clones of cells, including

cells with the capacity to respond to antigenic stimulation. Analysis of such clones might be expected to cast light on the cellular origins of antibody specificity.

SUMMARY

The relationship between hematopoietic colony–forming stem cells and cells in the thymus and lymph nodes of unirradiated mice has been investigated using a chromosome-marker technique. It was found that a high proportion of cells in the thymus may belong to the same clone as normal hematopoietic colony–forming cells. It was also found that cells belonging to the same clone as colony–forming cells may reach the lymph nodes, and that nodes containing such cells can participate in an immunological response against sheep red cells. Either the precursors of cells in thymus and lymph node are identical with hematopoietic colony–forming cells, or they are both descendants of a common precursor which has not yet been identified. The results are compatible with the view that cells of the hematopoietic system and the immune system may be derived from the same stem cell.

The authors are grateful to Miss Rosemary Wyncoll, Miss Judy Sheldon, Mrs. Clairellen Spencer, Mr. R. Course, Mr. A. Galberg, Mr. M. Kerr, and Mr. R. Kuba for technical assistance.

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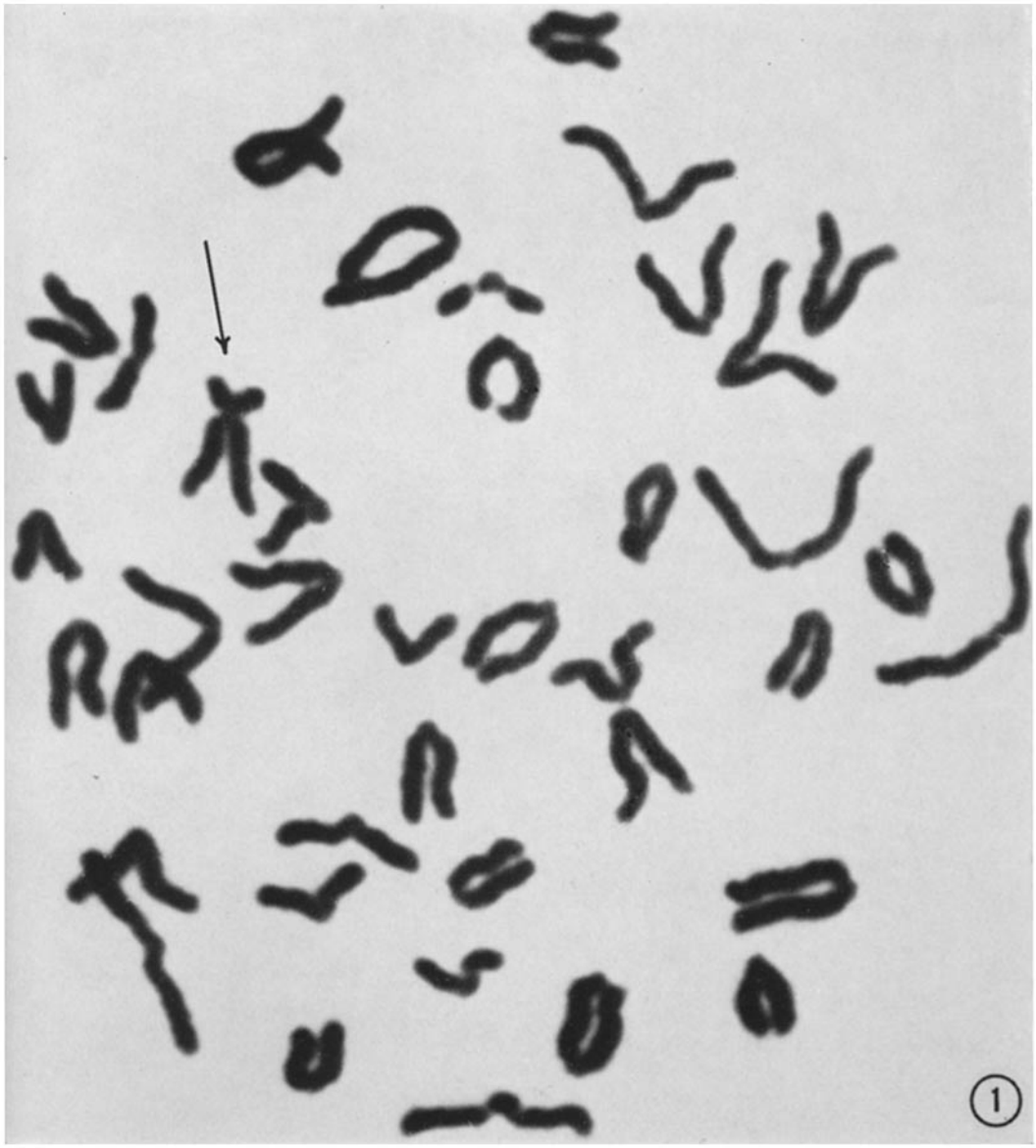
EXPLANATION OF PLATES

PLATE 61

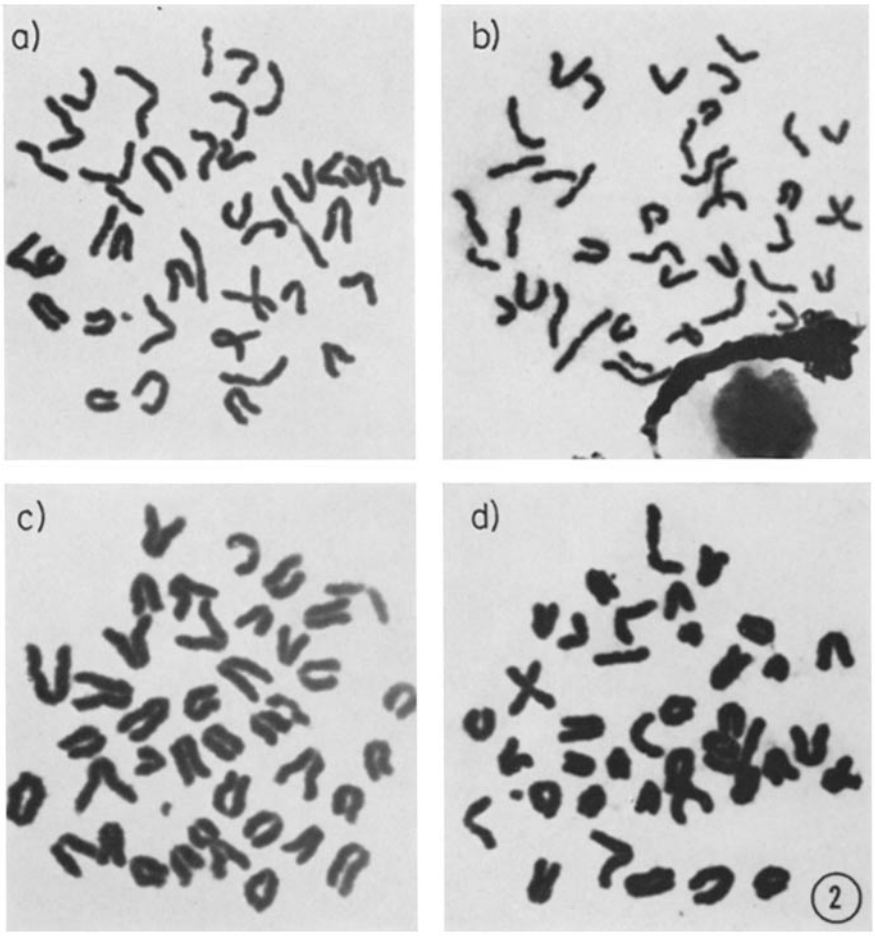
FIG. 1. Karyotype of a marked cell from W/W^v mouse 5 (Table I). The arrow indicates the metacentric marker chromosome. × 5800.



(Wu et al.: Hematopoietic cells and the lymphoid system)

PLATE 62

FIG. 2. Karyotypes of marked cells from W/W^v mouse 8 (Table I). Metaphases shown were obtained from: *a*, marrow (× 3000); *b*, spleen colonies (× 2500); *c*, thymus (× 4000); and *d*, lymph node (× 3700).



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