

BONE MARROW ORIGIN OF DECIDUAL CELL PRECURSORS IN THE PSEUDOPREGNANT MOUSE UTERUS*

By MARY KEARNS AND PEEYUSH K. LALA

From the Department of Anatomy, McGill University, Montreal, Canada H3A 2B2

Decidual cells constitute a distinctive cell class that appear in the endometrium of mammalian uteri during pregnancy, usually after the implantation of the blastocyst. These cells, along with other cells of the endometrial stroma, form a distinct layer referred to as decidual tissue. The decidual cell reaction can also be artificially induced in the hormonally primed pseudopregnant uterine endometrium by mechanical or chemical stimuli (1, 2).

Several biological functions have been attributed to decidual cells: (a) a nutritive role for the embryo postulated on the basis of their high glycogen content (3), (b) secretion of prolactin (4-6) and prostaglandins (7), and (c) maintenance of pregnancy by protecting maternal tissue from destructive invasion by trophoblast cells of the placenta (8) or by protecting the allogeneic fetoplacental unit from an immunological rejection by the mother (9, 10). For example, ectoplacental cones transplanted in the uterus of ovariectomized mice resulted in trophoblast cell invasion into the myometrial layer, which would otherwise be prevented in decidualized mice (8). Furthermore, primary allogeneic skin grafts transplanted into the decidualized uteri of pseudopregnant rats or rats in the preimplantation stage of pregnancy were found to survive significantly longer (usually to the time equivalent to term pregnancy) than those inserted into nondecidualized uteri (10). That orthotopically transplanted skin allografts in these rats did not survive longer appears to exclude the possibility of immunosuppression mediated by systemic hormones. Finally, materials released from decidual tissue in culture have been reported to demonstrate a suppressive function in the mixed lymphocyte reaction (11), and, similarly, extracts of decidual tissue have been associated with suppression of the antibody response of normal adult spleen cultures (12). The cell type of the decidua responsible for the suppression was not identified in either case.

Among a number of studies reported in the literature to examine the immediate origin of decidual cells, those using [³H]thymidine as a marker of cell proliferation (13-16) indicate that decidual cells arise from a proliferation and differentiation of endometrial stromal cells under the influence of ovarian hormones. However, the ultimate origin of the decidual cell precursors remains unknown. No information is available as to whether such precursors are local residents of the endometrium or migrants from elsewhere. In view of the possibility that decidual cells may play an immunological role in the maintenance of pregnancy, and in accordance with the knowledge that all cells of the adult immune system are derivatives of the bone

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marrow, the objective of our work was to determine whether or not decidual cells are ultimate descendants of marrow-derived cells.

Materials and Methods

Repopulation of Mice. To produce F_1 → parent chimeras, 15-wk-old CBA/J female mice ($H-2^k$) were lethally (950 rad) irradiated from a cobalt source (at a dose rate of 20 rad/min) and 4 h later repopulated intravenously with 10^7 bone marrow cells from 11-wk-old (CBA/J♀ × C57BL/6J♂) F_1 female ($H-2^{kb}$) mice. The use of F_1 semiallogeneic donors allowed a phenotypic identification of donor-derived cells while avoiding graft vs. host disease in the surviving chimeras. All mice were obtained from The Jackson Laboratory, Bar Harbor, ME. The drinking water of the repopulated mice was supplemented with gentamycin sulphate (Schering Canada, Point Claire, Quebec, Canada) at a concentration of 1 mg/liter.

Pseudopregnancy. Pseudopregnancy was initially attempted in young, ovariectomized CBA/J female mice to serve as a guide for pseudopregnancy induction in lethally irradiated, bone marrow-reconstituted mice. Because the ovaries of these animals were nonfunctional as a result of radiation damage, we hoped that they would respond as ovariectomized animals. The procedure reported by Miller and Emmens (17) was successful in both cases. This was provided by the following hormonal regimen: days 1, 2, and 3— injection of 0.1 μ g of 17- β -estradiol; days 4 and 5—no injection; days 6–12 or 13—daily injections of 6.7 ng estradiol and 1 mg progesterone. Each hormone was dissolved in Planter's Peanut Oil containing 5% ethanol by incubating for 45 min in a 37°C shaking water bath and injected subcutaneously in a 0.1-ml vol.

These mice did not show a normal estrous cycle before the introduction of the hormonal regimen, as indicated by daily vaginal smears. After the introduction of hormones, all animals moved into diestrus on day 7 and remained in diestrus as long as the injections of estrogen and progesterone were continued.

Decidual Reaction. A stimulus for the decidual reaction was introduced 6 h after the hormone injection on day 8 of the hormone regimen described above. 10 μ l of peanut oil was injected into the lumen of the right uterine horn. Mice were killed on days 13–14; i.e., 5–6 d poststimulation. Pilot experiments using ovariectomized mice indicated that the decidual reaction reached a maximum between 120 and 144 h poststimulation. This conclusion was based on changes in uterine weight as well as a histological evaluation of sections of uteri made at 72, 96, 120, and 144 h after introduction of the decidual stimulus.

Killing of Animals and Histological Preparation. Mice from which spleens and decidual nodules were collected for cell surface labeling were killed with an overdose of ether. All mice from which histological sections were made were killed by perfusion of phosphate-buffered saline followed by 2.5% glutaraldehyde (18) under sodium pentobarbital anesthesia. Tissues were embedded in Epon (Shell Chemical Co., NY) for making 1- μ m-thick sections. In some cases, tissues were fixed again in Bouin's fixative for paraffin embedding and making 5- μ m-thick sections.

Evaluation of Chimerism. Pilot studies revealed that long-term survival of the repopulated mice beyond 4 wk did not necessarily imply strong chimerism. Therefore, each mouse was evaluated separately for a phenotypic identification of donor-derived cells in the spleen and decidual nodules. These nodules (usually two to three in the stimulated horn, excluding the site of introduction of oil, and often one in the unstimulated horn, near the junction of the two uterine horns) were dissected out under a dissecting microscope and rinsed in ice-cold minimal essential medium (MEM)¹ containing 10% newborn calf serum (NCS), both purchased from Gibco Laboratories, Grand Island Biological Co., Grand Island, NY. Single-cell suspensions of these tissues were subjected to a sandwich-labeling of H-2 antigens, as described later, for a radioautographic identification of their surface phenotypes.

Preparation of Single-Cell Suspensions. Spleens were minced on a wire screen (80 mesh/in²) with iris scissors in ice-cold 10% NCS-MEM, subjected to a clump removal procedure by layering on NCS, erythrocyte lysis by treatment with 0.168 M NH_4Cl (19), and resuspended at

¹ Abbreviations used in this paper: NCS, newborn calf serum; NMS, normal mouse serum; MEM, minimum essential medium; MHC, major histocompatibility complex.

a concentration of 20×10^6 cells/ml of the same medium. Decidual nodules were minced with iris scissors and incubated in 0.3% collagenase made up in pH 7.4 Ca^{++} - and Mg^{++} -free phosphate-buffered saline containing 0.02% EDTA for 30 min in a 37°C shaking water bath. Cells were then spun down and subjected to an erythrocyte lysis and clump removal, as described above, before resuspending in ice-cold 10% NCS-MEM for surface labeling.

Cell Surface Labeling of H-2 Antigens. Surface H-2 antigens were detected and quantitated using a protein A sandwich technique. This was possible because the H-2 alloantibodies in the antisera employed contained sufficient protein A-binding IgG molecules. Single-cell suspensions were incubated with monospecific anti-H-2 sera or normal mouse serum (controls) at 1/20 dilution for 30 min at 4°C. Cells were washed twice through NCS and incubated in a 100- μl vol of medium with I^{125} -labeled protein A (sp act, 40 Ci/g; concentration 1 $\mu\text{g}/\text{ml}$) for 30 min at 4°C. Earlier studies had shown that I^{125} -protein A binds directly only to 1-3% of lymphoid cells (20) or macrophages (P. K. Lala, unpublished observation) in normal mice. Pilot experiments also showed that it does not bind directly to decidual cells, either in pregnant or pseudopregnant animals, and that an 1/20 concentration of the antisera gave optimum labeling conditions. Cells were then washed three times through NCS, resuspended in a drop of NCS, and smeared on slides coated with gelatin-chrome alum. Smears were fixed in methanol, processed for radioautography (21), and stained with Giemsa.

Sera. CBA/J mice were bled by intracardiac puncture to obtain normal mouse serum (NMS). Monospecific anti-H-2K^a and anti-H-2K^b sera were obtained through the courtesy of Dr. J. G. Ray of the National Institute of Allergy and Infectious Diseases, Bethesda, MD. Details pertaining to the antisera are presented in Table I. All sera were heat-inactivated at 56°C for 30 min and stored at -20°C until use. They were spun at 160,000 g in an air-driven ultracentrifuge for 30 min to remove protein aggregates before being used for cell surface labeling. Earlier studies in this laboratory employing these antisera failed to detect any non-H-2 related (e.g., antiviral) activity in these sera and have documented their binding specificity to various cell types by adsorption with H-2-matched targets (12, 22).

Radioiodination of Protein A. Staphylococcal protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) was radioiodinated using the chloramine T oxidation method (20), resulting in a sp act of 40 Ci/g.

Identification of Cells in Sections and Smears of Decidualized Tissue

SECTIONS. The morphological appearance of mouse decidual cells was evaluated through a sequential examination of the development of the decidual reaction in stimulated uterine horns of ovariectomized, hormone-primed mice at 72-144 h poststimulation. 5- μm -thick paraffin and 1- μm -thick Epon sections of uteri from these mice were compared with sections of uteri undergoing a decidual reaction at 4-14 d of pregnancy resulting from CBA and CBA matings. Paraffin sections were stained with periodic acid-Schiff (which stains the intracellular glycogen within decidual cells pink) or Masson's trichrome (which provides a green color to mucoproteins and collagen), and Epon sections were stained with toluidine blue. This analysis provided the morphological basis for the identification of decidual cells in smears and served as a guide for the optimum time of killing, i.e., the time at which the peak decidual response occurred.

SMEARS. For an unequivocal identification of decidual cells in smears, cell morphology isolated from pregnant animals was correlated with their histological appearance in semithin sections. In both preparations, decidual cells were identifiable as slightly vacuolated, occasionally binucleated cells of ovoid appearance. Their nuclei were eccentric in position, round or ovoid in shape, with uniformly dispersed chromatin containing one or more nucleoli. In smears

TABLE I
Details of Monospecific H-2 Antisera Used

Antiserum		Strains used for raising antisera		Cytotoxicity (50%) titer
Code	Haplotype specificity	Donor strain	Recipient strain	
D-23b	Anti-H-2K ^a	B10.A	(B10.D2 \times SJL)	700
D-33	Anti-H-2K ^b	B10.A5R	(B10.D2 \times A)	1400

stained with Giemsa, these cells showed a uniform blue cytoplasmic staining of basophilic nature. Their sizes, as determined with a calibrated eyepiece micrometer grid, ranged between 12 and 17 μm in diameter (average, 14 μm). Macrophages in smears were identified as highly vacuolated cells with abundant cytoplasm and characteristically convoluted nuclei. Their cytoplasmic staining was always lighter than decidual cells, and their sizes were highly variable, usually between 14 and 23 μm in diameter. Typical small lymphocytes (6–8 μm diameter) with round, purple-staining nuclei and a thin rim of blue cytoplasm, and granulocytes (7–9 μm Diam) with pink, lobulated nuclei and neutrophilic cytoplasm were also encountered in smears. Another minority cell type that remained unidentified was equal in size or slightly larger than the decidual cells and had acidophilic (pink), rarely vacuolated cytoplasm, and eccentric nuclei with uniformly dispersed chromatin. They were seen in smears of cell suspensions of decidual nodules in pseudopregnancy or decidua of early pregnancy, e.g., at day 8, but nearly disappeared by day 13 of pregnancy when the decidual reaction already reached its peak. These cells could possibly belong to the stromal cell class in the process of their transformation into decidual cells, but their definitive identification awaits further study. They were excluded from the decidual cell category in the present study.

Evaluation of Radioautographs. The threshold number of silver grains for positive labeling was determined from the knowledge of the grain count distribution on cell free areas of smears equivalent to the average size of the cell concerned (23). Based on the grain counts of >100 such cell-free areas, this threshold was taken as 6 grains for small lymphocytes and 11 grains for the decidual cells or macrophages. The number of silver grains was scored on 500 small lymphocytes in the case of the spleen and 100 decidual cells and the associated macrophages in the case of the decidual nodules from each mouse. Grain count distributions were then plotted for these cells.

Statistical Analysis. Some results were analyzed using the least-square method of linear regression. The degree of correlation was evaluated in each case from a computation of the correlation coefficient (r values).

Results

Cytology of Decidual Cells in Histological Sections and Smears. As stated in Materials and Methods, a careful light microscope evaluation of 5- μm -thick paraffin sections for cytochemical staining and 1- μm -thick Epon sections for morphological features revealed that the decidual cells seen in the decidual nodules of bone marrow-repopulated pseudopregnant mice were cytochemically and morphologically indistinguishable from those encountered in the nodules from pseudopregnant ovariectomized mice or decidualized endometrium of normal pregnant mice. Fig. 1 illustrates a section through a decidual nodule from a repopulated mouse uterus at 120 h poststimulation. A differential count of smears of cell suspensions from decidual nodules taken from 11 individual repopulated mice revealed the following incidence (mean \pm SE) of various cell types: decidual cells, 46.9 \pm 4%; macrophages, 18.4 \pm 1.9%; unidentified acidophilic cells, 23.1 \pm 3.6%; small lymphocytes, 6.6 \pm 1.8%; and polymorphonuclear neutrophils, 5.0 \pm 1.5%. In comparison, decidua basalis from day 13 of normal pregnancy yielded 83.9% decidual cells, 5.4% macrophages, 2.2% unidentified acidophilic cells, 4.3% small lymphocytes, 1.1% polymorphs, 1.1% nucleated erythroid cells, and 2% trophoblast cells. Representative cell types in smears from decidual nodules in repopulated mice are illustrated in Fig. 2.

Chimerism. The extent of chimerism within an individual cell type, namely, splenic lymphocytes, decidual cells, and macrophages within the decidual nodules, was evaluated in each individual repopulated mouse from the degree of labeling for the H-2K^b phenotype exclusively present on the donor-derived cells. The macrophages were intended to serve as positive control for chimerism within the decidual nodule,

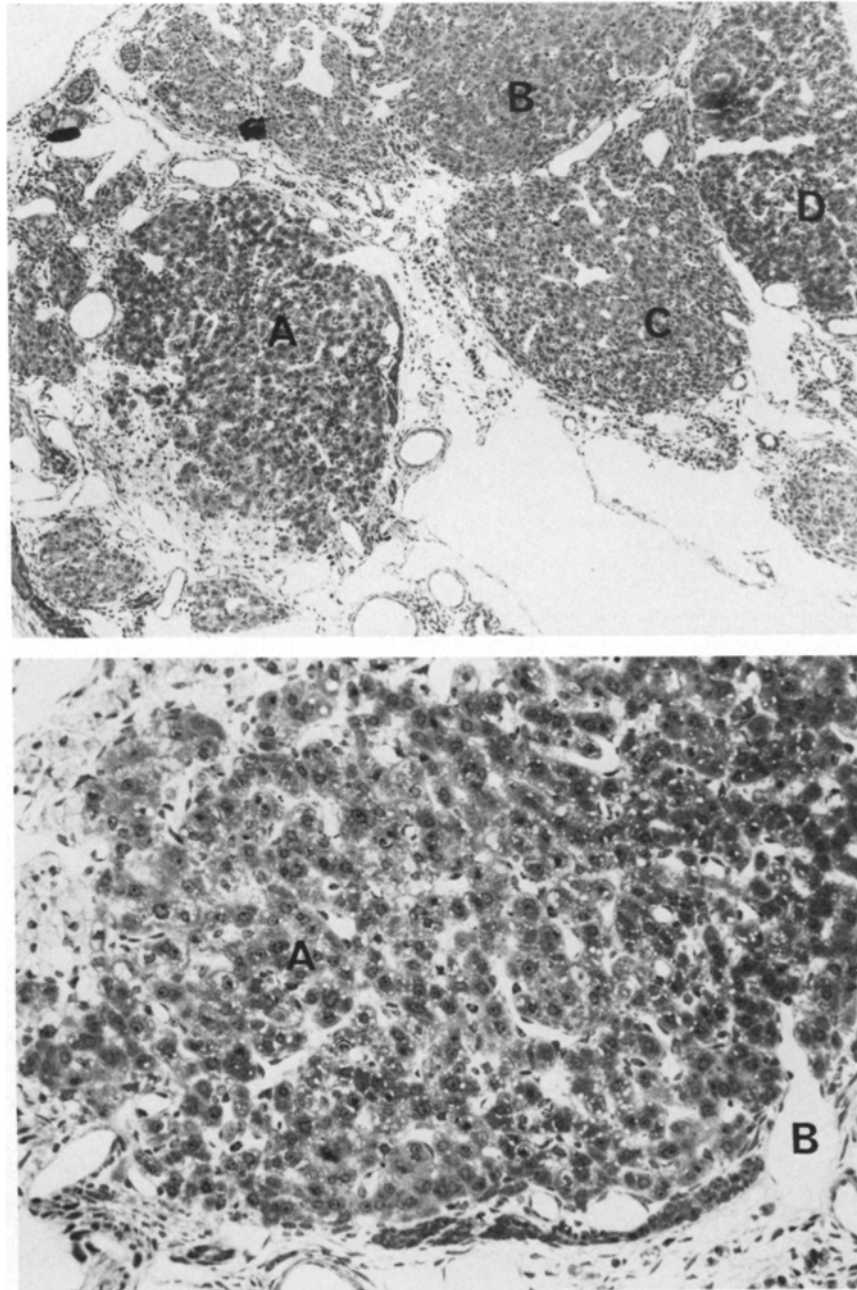


FIG. 1. Top: 1.0- μ m semithin Epon section of a decidua in a pseudopregnant repopulated mouse 120 h after decidual stimulation. Four microscopic nodules (A-D) are seen, each of which represents a cluster of transformed stromal cells. The section is stained with toluidine blue. $\times 120$. Bottom: higher magnification ($\times 360$) of part of a decidua nodule A seen in figure at the top. Decidual cells are uni- or binucleate, and often contain more than one nucleoli. Numerous capillaries are contained within the decidua. A, decidual cells; B, capillaries.

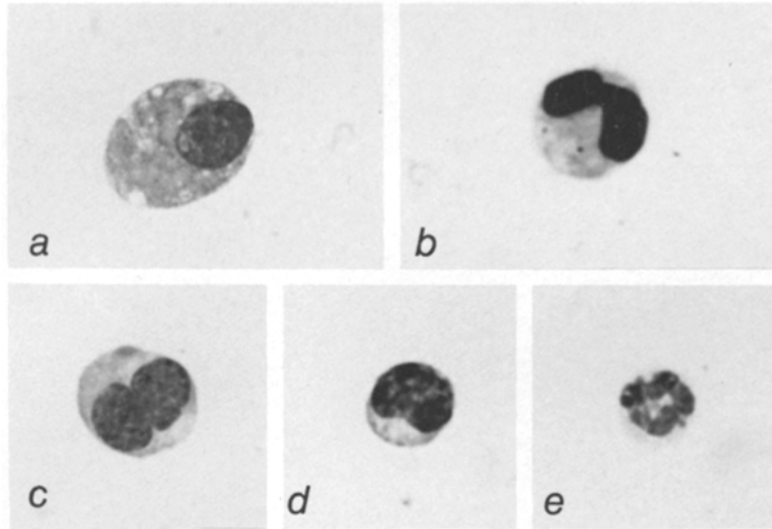


FIG. 2. Morphology of various cell types encountered in Giemsa-stained smears of decidual cell suspensions. A, decidual cell; B, macrophages; C, unidentified acidophilic cell (binucleate in this case); D, small lymphocyte; E, granulocyte. $\times 1,200$.

assuming they were derived from the bone marrow, as are macrophages elsewhere in the body (24). Labeling for the H-2^k phenotype (of the donor and host) served as the internal positive control for the labeling technique. The degree of labeling was expressed as the labeling index above background as well as the grain count distribution on the labeled cells, as compared with NMS controls. Labeling intensity, given by the number of silver grains, provided a relative measure of the antigen density among various cell types. Labeling for the K^b antigens on splenic lymphocytes in the individual repopulated mice served as an index of the extent of repopulation of the recipients by the donor type marrow cells, as opposed to repopulation by any surviving residual host type marrow cells. Fig. 3 illustrates some representative radioautographs under various labeling protocols.

Fig. 4 shows the labeling patterns (percentages of labeled cells in various grain categories) of splenic small lymphocytes of normal young adult (CBA/J \times C57BL/6J)F₁ mice when treated with anti-K^k, anti-K^b, or NMS. About 60–93% of these F₁ cells label for the K^k or K^b phenotype with the current labeling protocol (Table II). The same protocol has been noted to label 90–95% of parental strain CBA/J or C57BL/6J spleen cells in earlier studies in our laboratory (P. K. Lala, unpublished observation). A somewhat lower labeling of F₁ cells in the present case is considered to be a reflection of the lower density of both parental haplotype antigens inherited codominantly by the offspring. Thus, it is possible that the sensitivity of the current technique in detecting chimerism may have been slightly less than maximum. This would tend to result in false negatives rather than false positives.

Figs. 5–8 present the labeling patterns of the three cell types (splenic small lymphocytes, decidual cells, and macrophages in the decidual nodules) in four mice that represent typical results from a group of 11 repopulated mice in two separate experiments. Labeling indices (above background) for all experimental mice are

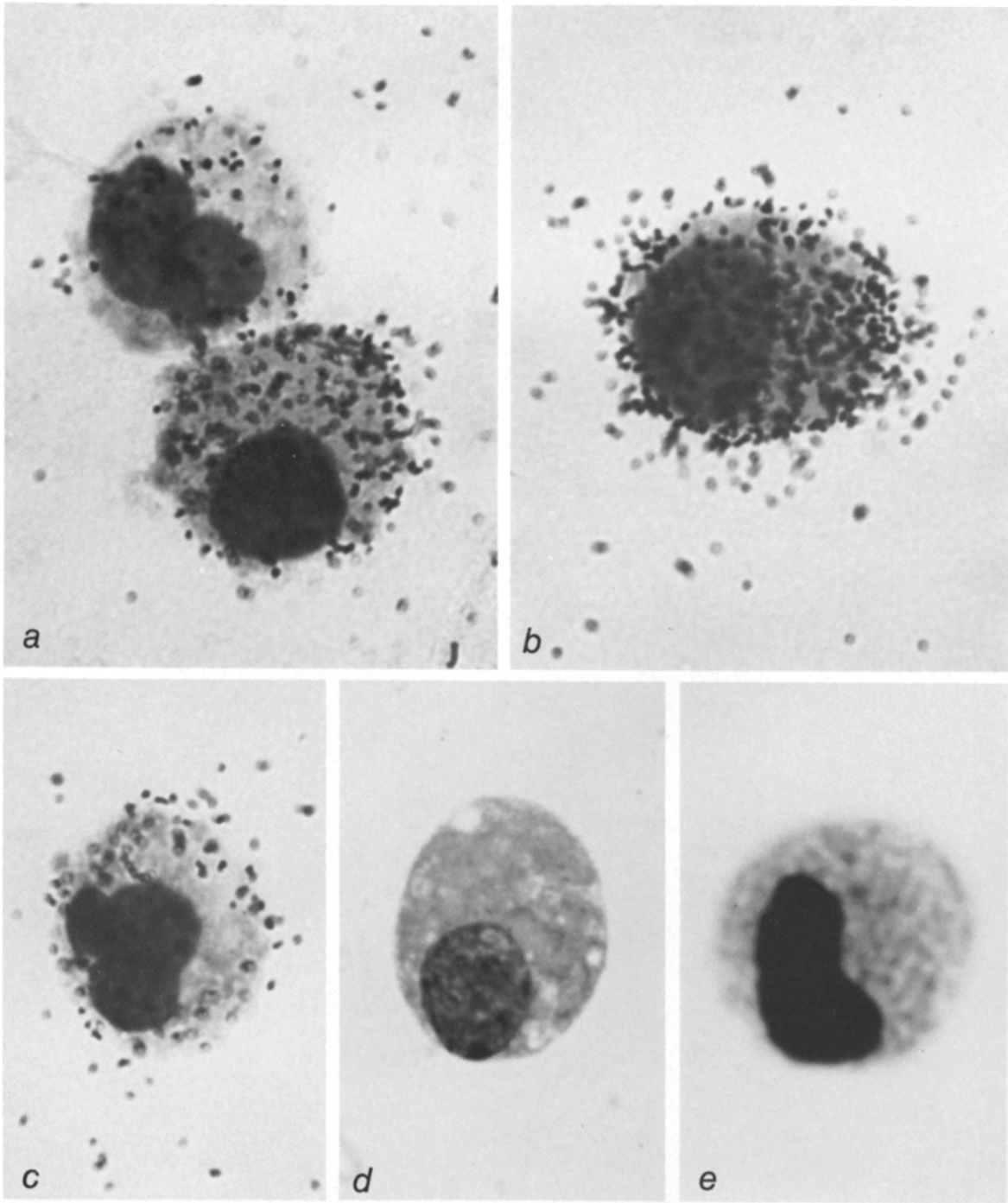


FIG. 3. Representative photomicrograph of radioautographs (stained with Giemsa) of cells obtained from decidual nodules of repopulated mice after various serum treatments followed by ^{125}I -labeled protein A. (a) decidual cell at the bottom and a macrophage at the top label heavily with anti-H-2K^b serum, i.e., for the donor phenotype. (b) decidual cell, and (c) macrophage label heavily with anti-H-2K^k serum, i.e., for the donor or the host phenotype. (d) decidual cell, and (e) macrophage exhibit no labeling after NMS treatment. $\times 2,000$.

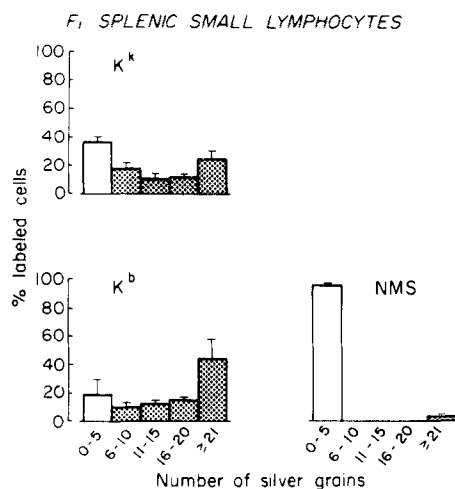


FIG. 4. Labeling patterns (mean \pm SE) for splenic small lymphocytes of three normal (CBA/J ϕ \times C57BL/6J δ)F₁ mice exposed to anti-H-2K^k, anti-H-2K^b, or NMS. Both antigens are expressed codominantly as indicated by the grain count density.

TABLE II
H-2-labeling Patterns in Individual Animals

Series	Mouse	Uterine weight ratio*	Percent labeled above background					
			Decidual cells		Macrophages		Spleen	
			K ^k	K ^b	K ^k	K ^b	K ^k	K ^b
1‡	1.1	5.1	81.3	29.9	55	37.3	95.4	49.6
	1.2	8.4	79.4	36.8	86.7	59.1	85.6	69.5
	1.3	6.2	90.5	41.3	91	51.9	67.8	80
	1.4	6.6	96.8	70.2	100	68.6	82.8	46.4
	1.5	11	87.3	61	95.2	50	94.2	25.2
	1.6	4.3	94.5	18.9	95.2	40	83.4	81.8
	1.7	6.5	60	2.8	85.2	12.3	61.8	2.8
	1.8	4.3	99.2	5.1	97.6	16.7	88	9.5
	1.9	5.1	67.3	17.1	50	17	51.5	0
2§	2.1	7	65.5	73.9	60	78.7	76.6	68.8
	2.2	4.3	100	7.5	100	17.3	85.4	27
F ₁ (a)							58.8	58.4
F ₁ (b)							69.0	93.0
F ₁ (c)							62.8	92.6

* Stimulated horn/control horn.

‡ Used 4 wk after reconstitution.

§ Used 10 wk after reconstitution.

presented in Table II. This table also shows the relative extent of decidual response as reflected in the uterine weight ratio.

Labeling index resulting from NMS treatment followed by ¹²⁵I-protein A ranged for the most part between 0 and 10% for all cell types concerned (Figs. 5-8), representing weakly labeled cells with low grain densities, marginally above back-

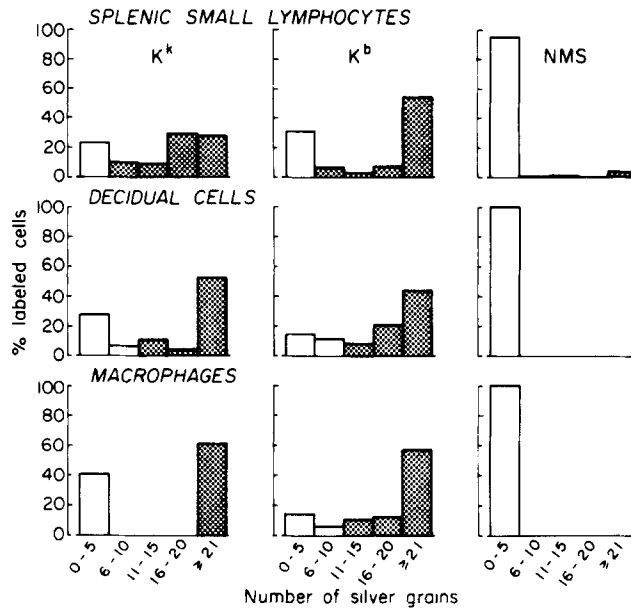


FIG. 5. H-2-labeling patterns for splenic small lymphocytes, decidual cells, and macrophages within the decidual nodules for bone marrow-repopulated mouse 2.1 (Table II). Shaded areas represent positive labeling above background. Good chimerism is indicated by the K^b-labeling pattern in all three cell types.

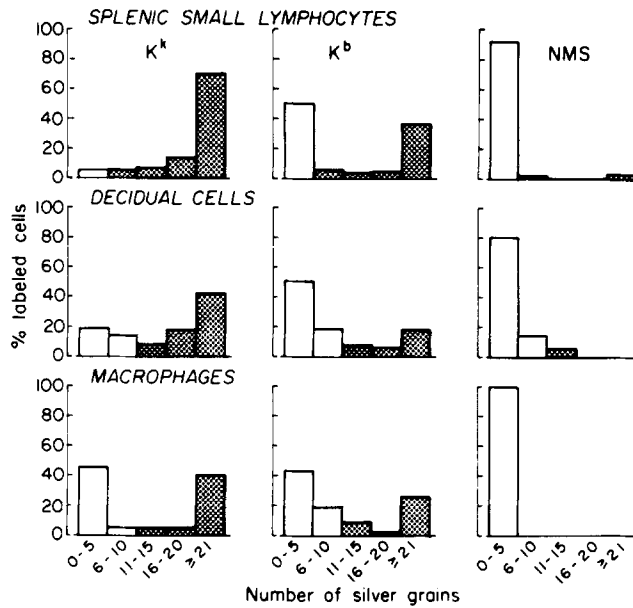


FIG. 6. Labeling patterns for splenic small lymphocytes, decidual cells, and macrophages within the decidual nodules for bone marrow-repopulated mouse 1.1 (Table II). A fair degree of chimerism is indicated by the K^b-labeling pattern in all three cell types.

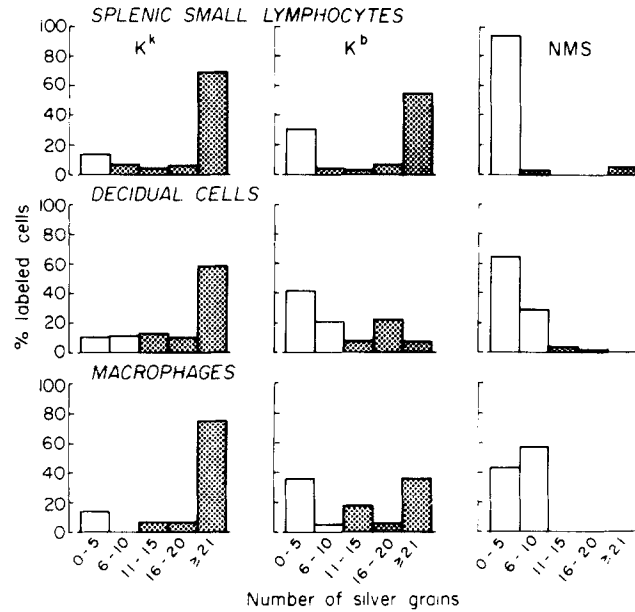


FIG. 7. H-2-labeling patterns for splenic small lymphocytes, decidual cells, and macrophages within the decidual nodules for bone marrow-repopulated mouse 1.2 (Table II). Good chimerism is present on the splenic lymphocytes and macrophages within the decidual nodules, and fair chimerism is evident on decidual cells, as indicated by the K^b-labeling pattern.

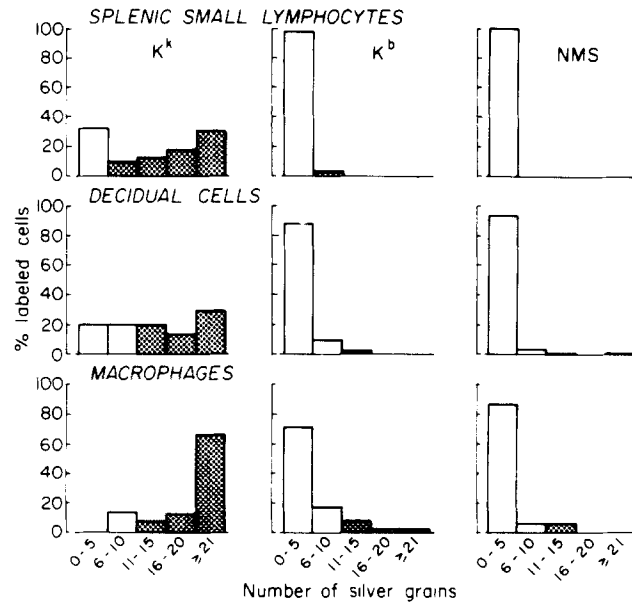


FIG. 8. H-2-labeling patterns for splenic small lymphocytes, decidual cells, and macrophages within the decidual nodules for bone marrow-repopulated mouse 1.7 (Table II). No evidence of chimerism is present on any of the three cell types.

TABLE III
 Statistical Parameters of Two-Variable Linear Regression Analysis of K^b -labeling Indices under Various Conditions

Condition	x	y*	b_1	b_0	r_{\ddagger}
Uncorrected for NMS	Decidual cells	Lymphocytes	0.56	23.32	0.47
Uncorrected for NMS, excluding chimeras 1.5, 1.6	Decidual cells	Lymphocytes	0.83	12.98	0.73
Corrected for NMS	Decidual cells	Lymphocytes	0.55	21.26	0.45
Corrected for NMS, excluding chimeras 1.5, 1.6	Decidual cells	Lymphocytes	0.87	11.67	0.72
Uncorrected for NMS	Decidual cells	Macrophages	0.82	13.53	0.93
Corrected for NMS	Decidual cells	Macrophages	0.75	9.86	0.80
Uncorrected for NMS	Macrophages	Lymphocytes	0.97	2.41	0.72
Uncorrected for NMS, excluding chimeras 1.5, 1.6	Macrophages	Lymphocytes	1.02	-1.23	0.84
Corrected for NMS	Macrophages	Lymphocytes	0.88	9.56	0.67
Corrected for NMS, excluding chimeras 1.5, 1.6	Macrophages	Lymphocytes	0.96	5.25	0.82

* $y = b_0 + b_1x$.

\ddagger Regression coefficient.

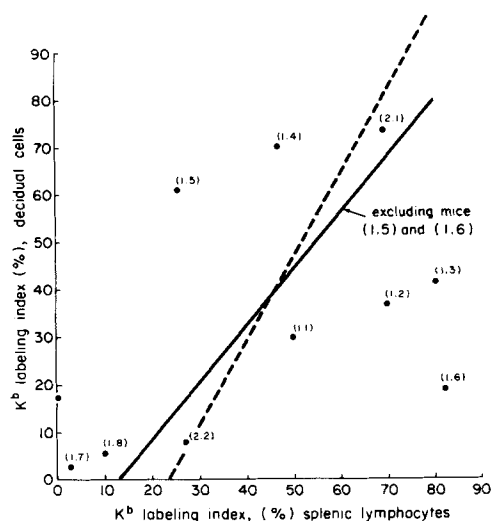


FIG. 9. Two-variable linear regression analysis comparing chimerism (as indicated by the K^b -labeling index) on decidual cells vs. splenic lymphocytes. When all repopulated mice are included in the computation of the correlation coefficient, the relationship appears weak: $r = 0.47$ (---). If, however, mice 1.5 and 1.6 are excluded from the analysis because they represent extreme departures from the normal relationship and fall just outside the 95% confidence intervals of the regression line, the correlation becomes strongly positive: $r = 0.73$ (—).

ground. Because the incidence of cells labeled with ^{125}I -protein A alone was 1–3% for lymphocytes and macrophages and 0% for decidual cells (data not shown), additional weak labeling after NMS treatment in some cases may be a result of autoantibody type molecules in mouse sera or binding of Fc receptors to serum Ig aggregates in which some binding sites for protein A remained accessible. The latter possibility is less likely, as all sera were spun to remove aggregates before use. This non-H-2-related

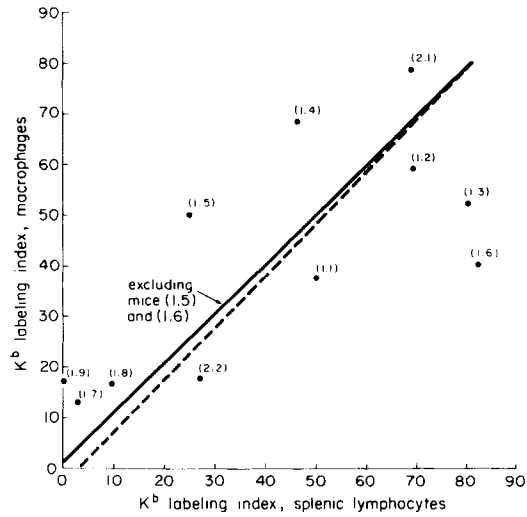


FIG. 10. Two-variable linear regression analysis comparing chimerism (as indicated by the K^b -labeling index) on macrophages within the decidual nodules vs. splenic lymphocytes. The correlation coefficient is strong ($r = 0.72$) when all mice are included (---), but improves further to 0.84 when mice 1.5 and 1.6 are excluded from the analysis (—).

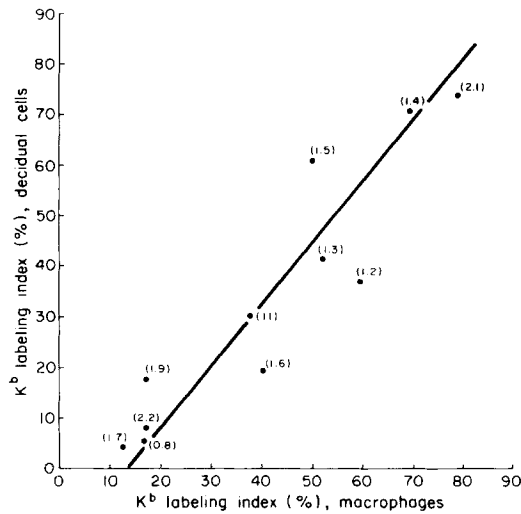


FIG. 11. Two-variable linear regression analysis comparing the K^b -labeling index of decidual cells vs. macrophages. A computation of the correlation coefficient of these two variables indicates a very strong positive correlation: $r = 0.93$ (—).

binding was nevertheless taken into consideration while evaluating H-2-related labeling using anti-H-2 sera in the individual repopulated mice.

Good labeling for K^k antigens (pertaining to the donor or the host) was noted in all three cell types of all mice tested (Figs. 5-8, Table II). For example, the incidence of labeled small lymphocytes varied from 52 to 95% in the spleen, where 70% of the labeled cells on the average showed a labeling intensity of >16 grains per cell. The incidence of labeled macrophages within the decidual nodules ranged between 50

TABLE IV
Degree of Chimerism in Various Cells of Repopulated Mice

Mouse	Splenic lympho- cytes	Degree of chimerism*	
		Macrophages	Decidual cells
1.1	Fair	Fair	Fair
1.2	Good	Good	Fair
1.3	Good	Fair	Fair
1.4	Fair	Good	Good
1.5	Low	Fair	Good
1.6	Good	Fair	Low
1.7	None	None	None
1.8	None	Poor	None
1.9	None	Poor	Poor
2.1	Good	Good	Good
2.2	Low	Poor	Poor

* Based on K^b -labeling index minus NMS-labeling index. >50%, good; 25–49%, fair; 15–24%, low; 5–14%, poor; <5%, none.

and 100% and this was 60–100% for decidual cells. 80–90% of the labeled macrophages or decidual cells had at least 16 grains per cell. Thus, surface labeling patterns for K^k antigens on decidual cells and macrophages contained within the decidual nodules was found to parallel the labeling patterns in the splenic lymphocytes in all 11 repopulated mice.

The degree of labeling for K^b among splenic small lymphocytes (Figs. 5–8, Table II), reflecting the degree of repopulation by the donor type marrow, varied substantially from mouse to mouse. Hence, the K^b labeling pattern of decidual cells in each mouse was evaluated in the context of K^b labeling patterns of splenic lymphocytes and macrophages within the decidual nodules. To evaluate the relationship of the degree of K^b labeling between any two cell classes, a two-variable linear regression analysis was performed, and the correlation coefficients were computed in each case. These data are presented in Figs. 9–11 without any correction for NMS binding of these cells. When these data were plotted after correction for NMS binding, very little or no change in the statistical parameters was noted (see Table III) and thus are not presented in the form of figures.

When all repopulated mice are included in the regression analysis, the correlation between decidual cell chimerism and splenic lymphocyte chimerism appeared weak ($r = 0.47$), as seen in Fig. 9. However, if one excludes mice 1.5 and 1.6, which represent extreme departures in this relationship and fall just outside the 95% confidence intervals of the regression line, there is an enormous improvement in the positive correlation of chimerism between the two cell types ($r = 0.73$). Fig. 10 represents the relationship of chimerism in macrophages to that of splenic lymphocytes. In this case, the positive relationship is strong ($r = 0.72$) even when all mice are included. It improves further ($r = 0.84$) when, again, the same two mice (1.5 and 1.6) are excluded from the analysis. A very strong positive correlation ($r = 0.93$) is noted between decidual cell labeling and labeling on macrophages, as seen in Fig. 11.

To provide a simple means of comparing the extent of chimerism among the three cell classes of the repopulated mice, chimerism was arbitrarily graded into five ranks (Table IV), based on the K^b labeling index over and above the NMS labeling index:

good ($\geq 50\%$), fair (25–49%), low (15–24%), poor (5–14%), and none ($< 5\%$). It is clear that 9 out of 11 mice (i.e., excluding 1.5 and 1.6) showed a good match of chimerism (falling within the same rank or varying by only one rank) amongst all three cell types, whereas by the same criterion a good match between splenic lymphocytes and macrophages or macrophages and decidual cells was seen in all mice. On no occasion was complete absence of chimerism seen in one cell class associated with fair or good chimerism in another cell class. In five mice showing fair to good chimerism in the splenic lymphocytes as well as macrophages and decidual cells, the intensity of K^b labeling patterns as given by grain count distributions also showed some parallelism. Three of these cases have been presented in Figs. 5–7.

Discussion

This study examined the possible bone marrow origin of decidual cell precursors by an examination of the H-2 phenotype of decidual cell in pseudopregnant bone marrow chimeras using a highly sensitive and specific immunolabeling technique followed by radioautography. To apply this approach, the morphological identity of decidual cells in smears was first unequivocally established from a cytochemical and morphological correlation of decidual cells in sections with those in smears during the peak decidual response in pregnancy. Identical morphological features were confirmed in decidual nodules produced in pseudopregnant ovariectomized mice as well as bone marrow-repopulated mice. Great care was taken to exclude macrophages from the decidual cell class on the basis of distinctive morphological features as described in Materials and Methods. This was an important prerequisite for an elimination of false-positive results when testing the possible bone marrow origin of decidual cells. Similarly, a small minority of unidentified acidophilic cells encountered in the smears of decidual nodules (which may represent transitional forms between stromal cells and decidual cells) were also excluded.

A detailed examination of the H-2-labeling patterns of various cell classes reveals several important features. First, there was a good positive correlation of the extent of chimerism in the splenic lymphocytes with that in the macrophages within the decidual nodules in all repopulated mice. In no case was chimerism seen in the macrophages in the absence of chimerism in the splenic lymphocytes and vice versa. These findings confirm our assumptions that macrophages within the decidual nodules are the progeny of derivatives of the bone marrow, as are macrophages elsewhere in the body.

Second, there was also a good correlation of the extent of chimerism in the splenic lymphocytes with that in the decidual cells in all but two repopulated mice. 6 out of 11 mice that showed fair to good chimerism in the spleen also showed similar chimerism in the decidual cells, except one in which this was low. The remaining five mice that had low or insignificant levels of chimerism in the spleen also showed similar levels of chimerism in the decidual cells, except one in which this was higher. Again, a complete absence of chimerism in the splenic lymphocytes was never associated with good chimerism in the decidual cells and vice versa. These results lead us to conclude that decidual cells in pseudopregnant mice are ultimate descendants of the bone marrow. The key finding underlying this conclusion is that no explanation other than chimerism can be afforded for positive K^b labeling above the NMS labeling (which was low) in the decidual cells. An absence of chimerism in the spleen of three

mice, which may serve as negative controls, also gave negative results for the decidual cells. Two possible reasons may be offered to account for the departure from a positive correlation of the extent of K^b labeling in the spleen with that in the decidual cells in two mice. First, the migration patterns of the precursors of the splenic lymphocytes from the bone marrow during the weeks between repopulation and killing may have differed from the migration patterns of the putative precursors of the decidual cells from the bone marrow in these two animals. This may not be surprising since it is very likely that these precursors are different cells, albeit both marrow-derived. Second, the current results cannot exclude the possibility that there may exist two kinetically distinct populations of decidual cells: a majority whose precursors are migrants from the bone marrow, and a minority whose precursors are derived from local, resident cells. An alteration in the balance between these two subpopulations may have led to a discordance between the K^b labeling pattern of the spleen and that of the decidual cells. Further work is needed to investigate these possibilities.

Finally, the excellent positive correlation between the K^b-labeling patterns of the decidual cells and macrophages within the decidual nodules reinforces the conclusion that decidual cell precursors are derived from the bone marrow. This also raises the unconfirmed but nevertheless interesting possibility that both cell classes are derived from the same precursors, namely, monocytes. Additional work is needed to substantiate or exclude this hypothesis.

The identity of the bone marrow-derived cells which migrate to the uterus and give rise to the stromal antecedents of the decidual cells remains to be determined. Theoretically, this may be the multipotential hemopoietic stem cell or any of its differentiated progeny. It is highly likely that migration of putative decidual cell precursors from the bone marrow to the endometrium of the uterus is under hormonal control. The accumulation of leukocytes in the uterine endometrium has been shown to exhibit predictable patterns which are associated with the estrous cycle, most likely under the influence of hormonal surges (25, 26). It is possible that in the current experimental situation the precursor cell migration occurred mostly during the period of hormone administration for the induction of pseudopregnancy rather than before this period, soon after repopulation with bone marrow cells.

Because bone marrow radiation chimeras cannot be impregnated by the normal mating process because the radiation damage to the ovary has rendered them anovular, pseudopregnancy rather than normal pregnancy had to be employed in this study. The question remains open as to whether decidual cell precursors are also derived from the bone marrow during normal pregnancy. Attempts are currently being made in our laboratory to produce pregnancy in hormone-primed bone marrow chimeras by a transfer of blastocysts from normal pregnant mice.

The ultimate functional significance of our findings remains unknown. Our investigation was inspired by the possibility that decidual cells may play an immunological role in the maintenance of allogeneic pregnancy. Preimplantation mouse blastocysts implant in the endometrium and grow successfully despite their expression of H-2 antigens (27). Similarly, studies from our laboratory have unequivocally demonstrated the presence of class I major histocompatibility complex (MHC) (H-2K and D) antigens on mouse trophoblast cells both in single cell preparations (19) as well as *in situ* on their sinusoidal face exposed to the maternal blood,² yet they do not evoke

² S. Chatterjee-Hasrouni and P. K. Lala. 1982. Localization of paternal H-2K antigens on murine trophoblast cells *in vivo*. *J. Exp. Med.* In press.

an immunological rejection by the mother. Whether the poor immunogenicity of trophoblasts owes to the paucity of class II MHC (Ia) antigens on their surface (28), or is a result of some local immunosuppressor mechanism, remains undetermined. Decidual cells that appear at the implantation site and remain in vicinity of the fetomaternal interface (at least up to day 13 of gestation in mice) may be one source of immunosuppressor molecules. Some cells in the decidual layer (29) or their products (11) have been reported to be capable of suppressing in vitro alloreactivity. However, the precise morphological identity of the suppressor cells in the decidual tissue has not been established. Furthermore, decidual cells have been reported to express Thy-1 antigens and Fc receptors (30, 31), and it has been proposed that their Fc receptors may be instrumental in localizing the blocking antibodies directed against paternal type antigens (32). Finally, it is also possible that a bone marrow origin of decidual cell precursors may imply functions distinct from immunological ones.

Summary

Decidual cells are considered to be the endproduct of a hormonally induced transformation of endometrial stromal cells of the uterus. However, the source of these precursors remains unknown. This study evaluated the possibility of their bone marrow origin by an examination of the H-2 phenotype of decidual cells in pseudopregnant bone marrow chimeras. These chimeras were produced by repopulating lethally irradiated CBA/J female (H-2^k) mice with bone marrow from (CBA/J × C57BL/6J)F₁ female (H-2^{kb}) mice. Pseudopregnancy was produced with a hormonal regimen followed by an oil-induced decidual stimulus. Chimerism was evaluated radioautographically by an identification of the donor-specific K^b phenotype on cells with an immunolabeling technique with monospecific anti-H-2 serum followed by radioiodinated protein A. The extent of chimerism as indicated by the degree of K^b labeling on decidual cells as well as macrophages contained within the decidual nodules was quantitatively compared with that seen on splenic lymphocytes.

Fair to good chimerism, as reflected by labeling for the donor-specific marker (K^b), was seen on splenic lymphocytes and macrophages within the decidual nodules in 6 out of 11 animals. A similar level of chimerism was detected on decidual cells in all but one of these six, in which case this was low. One animal showed low chimerism in the spleen but good chimerism on the decidual cells. The remaining four mice were nonchimeric for all three cell types. These results indicate that decidual cells and macrophages appearing within the decidual nodules of pseudopregnant mice are ultimate descendants of bone marrow cells.

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