ROLE OF CELL CONTACTS FOR DIFFERENTIATION OF PRECURSOR CELLS OF HEMATOPOIETIC STROMA IN LONG-TERM BONE MARROW CULTURES

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For many years it was impossible to obtain long-term bone marrow cultures by explantation of hematopoietic cells in the form of unicellular suspensions. Clonogenic stromal precursors of bone marrow formed colonies of fibroblast-like cells in cultures, but hematopoiesis was quickly exhausted [2]. Recently, cultures of mouse bone marrow have been successfully obtained in which hematopoiesis was maintained for many weeks through the functioning of a composite underlayer of adherent cells. One of the chief factors enabling a properly functioning underlayer to be formed is explantation of bone marrow in the form of fragments and not a unicellular suspension [4]. It can be tentatively suggested that a factor of great importance for differentiation of stromal precursors is the way they are packed at the time of explantation. This problem was studied experimentally in the investigation described below.

EXPERIMENTAL METHOD

Female C57BL/6 mice aged 8-20 weeks were used. To obtain chimeras, mice were irradiated with ^{137}Cs γ-rays in a dose of 10.5 Gy and were restored by intravenous injection of bone marrow from syngeneic donors (one-third femoral equivalent). The bone marrow culture was grown in plastic flasks, the bottom of which had an area of 25 cm^2, at 33°C. Fisher's medium with 25% serum (two parts horse and one part embryonic calf), L-glutamine, hydrocortisone (10^{-7} M), were used. Bone marrow was explanted either as fragments, flushed out by a jet of complete medium (10 ml) from the femur into the flask, or as a cell suspension obtained by repeated passage of the bone marrow through a syringe with No. 21 needle, also in an amount of one femoral equivalent per flask in 10 ml medium. Half of the medium was replaced by fresh every week. A "wound" was inflicted by removing the underlayer from half of the area of the bottom of the flask by means of a rubber "policeman." The number of stromal precursor cells able to transfer the hematopoietic microenvironment was determined by implantation of the underlayer of the cultures removed by the "policeman" beneath the renal capsule of syngeneic recipients, either irradiated or chimeras. The size of the foci of ectopic hematopoiesis thus formed was determined 1 month after explantation from the number of nucleated hematopoietic cells which they contained.

EXPERIMENTAL RESULTS

On explantation of bone marrow in the form of fragments, a characteristic composite underlayer consisting of fibroblast-like cells, giant fat cells, histiocyte-macrophages, and endothelial cells, arranged in many layers, formed quickly, in the course of 1-2 weeks. Characteristic sites of active hematopoiesis, in which many hematopoietic cells were present, could be seen on the underlayer (Fig. 1). The culture had a completely different appearance when formed by explantation of a cell suspension (Fig. 2). The cells grew in the form of a monolayer, consisting chiefly of dispersed histiocyte-macrophages and fibroblast-like cells. Colonies of densely packed "fibroblasts," among which macrophages were often found, were fairly numerous. Hematopoiesis was not observed, either in colonies or in the monolayer.

Consequently, separation of bone marrow cells leads to changes in their differentiation. Stromal precursors under these conditions either form clones of relatively simple structure or differentiate into separately lying cells. Meanwhile, they do not complete the whole diversity of cell differentiations that would lead to the formation of the complex structures of the

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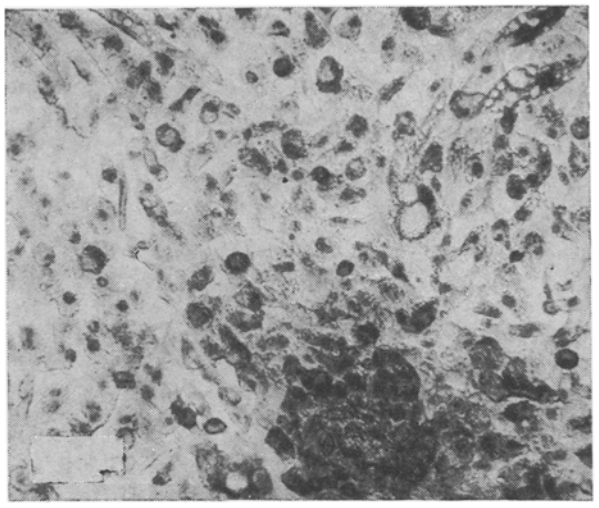

Fig. 1

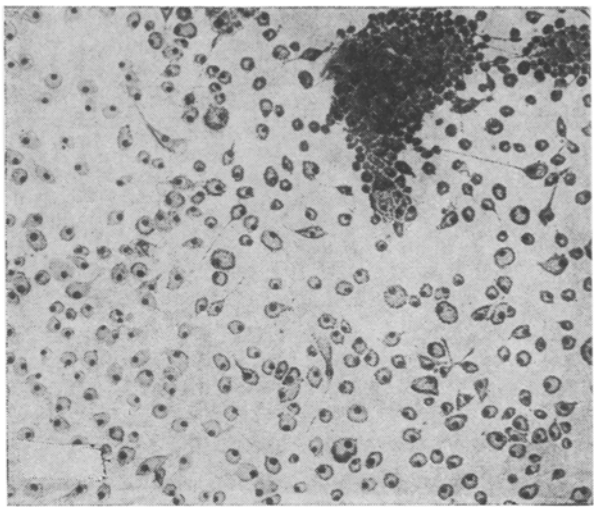

Fig. 2

Fig. 1. Region of hematopoiesis in 4-week culture of mouse bone marrow. Cells characteristic of the composite monolayer can be seen. Here and in Figs. 2-4, staining by Giemsa's method, 100 ×.

Fig. 2. Underlayer in 4-week bone marrow culture obtained by explantation of cell suspension. Colony of fibroblasts and macrophages can be seen.

TABLE 1. Transfer of Hematopoietic Microenvironment by Cultures of Bone Marrow Fragments or Suspensions of Bone Marrow Cells

No. Expt	Method of explantation	Duration of culture, weeks	Size of focus of ectopic hematopoiesis ($\times 10^{-6}$)			
			No. of cultures	intact recipients	No. of cultures	Irradiated recipients
1	Suspension	7	4	Foci absent	—	—
2	Suspension	5	2	Same	2	0,4
	Fragments	5	2	9,3	2	55,0
3	Suspension	4	2	Foci absent	2	0,5
	Fragments	4	2	8,0	1	7,6

TABLE 2. Size of Foci of Ectopic Hematopoiesis Formed by Regenerating or Intact Sublayer from 3-Week Bone Marrow Cultures (wound inflicted after 1 week in culture)

Underlayer	Size of focus of ectopic hematopoiesis ($\times 10^{-6}$)			
	No. of cultures	intact recipients	No. of cultures	irradiated recipients
Uninjured part	4	3,8	4	8,6
Regenerating part	4	1,2	4	5,0

hematopoietic microenvironment in the culture. A less likely suggestion is that separation of the cells leads to death of the corresponding precursors, if only because the separated cells can construct a hematopoietic microenvironment in the culture under special conditions (in collagen gel) [5].

It was shown previously that the hematopoietic microenvironment is transferred during implantation of bone marrow by stem cells of the hematopoietic stroma, known as HMTU (hematopoietic microenvironment-transferring units) [1]. The question naturally arises, are HMTU present in the underlayer of stromal cells which can or cannot maintain hematopoiesis. Cells in the underlayer of cultures of Dexter type contain HMTU and transfer the microenvironment when transplanted back from culture into the animal. In the case of implantation beneath the kidney capsule, the underlayer of cultures of bone marrow cell suspensions, the microenvironment was not transferred and no focus of ectopic hematopoiesis was formed. Consequently, separation of cells of explanted bone marrow leads not only to a change in the type of differentiation of the stromal precursor cells, as a result of which an underlayer is formed which cannot maintain hematopoiesis, but also to exhaustion of the HMTU, most probably on account of their irreversible, i.e., "suicidal" differentiation (Table 1). An alternative explanation may be that although

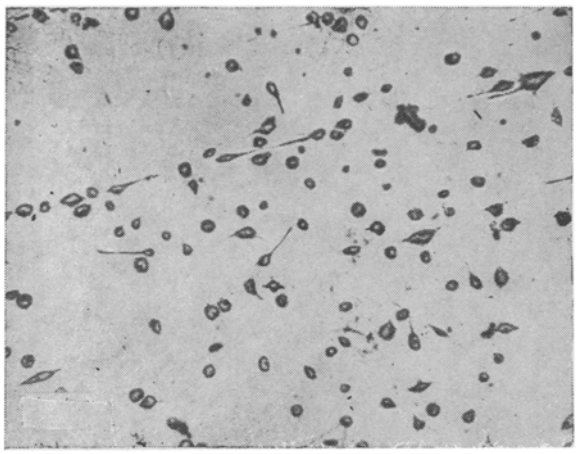

Fig. 3

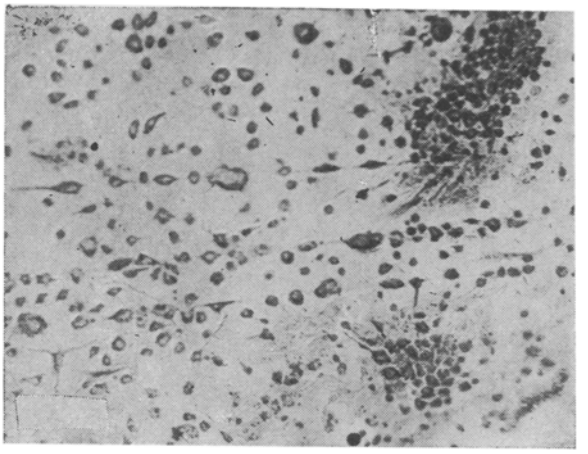

Fig. 4

Fig. 3. Underlayer at site of infliction of "wound" in 3-week culture 1 week after injury.

Fig. 4. Underlayer and site of infliction of wound in a week-old culture 2 weeks after injury.

HMTU are preserved in the underlayer constructed by a suspension of tumor cells, their ability to transfer the microenvironment is blocked by the absence of their essential structural organization. In this case, it is possible that other methods of transfer of the underlayer back into the animal, enabling the necessary intercellular interactions to take place, could prove effective.

The data show the importance of intercellular interaction for the fate of explanted bone marrow stromal cells. The question arises: what is the role of intercellular interaction in an already formed underlayer of adherent cells. On explantation of bone marrow in the form of fragments in which intercellular contacts are preserved, stromal precursors, which at the time of explantation occupied a negligible fraction of the area of the bottom of the flask, creates the characteristic structural organization over the whole area of the bottom of the flask, i.e., they migrate for very long distances for cells, without losing the necessary intercellular contacts (or recreating them constantly).

Does the underlayer, when formed, still have this ability? To study this problem, a "wound" was inflicted on a 3-week culture exhibiting good hematopoiesis. After 1 week, only single fibroblast-like cells and macrophages, which had migrated into it, could be observed at the site of the wound (Fig. 3). No composite underlayer was formed in the wound. Quite different results were obtained when the wound was inflicted on an underlayer still in the process of formation, after 1 week in culture, when the underlayer was still spread over the whole of the flask, it contained no giant fat cells, it was not so stratified, and areas of hematopoiesis were only just beginning to be formed in it. By 1 week after wounding, it was so completely invaded by a characteristic underlayer that the site of injury could be recognized only by the presence of fibroblast-like cells stretched out along scratches on the bottom of the flask (Fig. 4). The underlayer regenerated not only morphologically, but also functionally — it supported normal hematopoiesis and created cells capable of building the microenvironment on retransplantation back into the animal (Table 2).

The results of this investigation demonstrate the great importance of intercellular contacts for differentiation of stromal precursors while they are forming the hematopoietic microenvironment. The ability of stromal precursors to preserve intercellular contacts in the course of formation of a new hematopoietic territory in culture, just like their loss of these properties after formation of a functioning underlayer, are more surprising still.

Hematopoietic stem cells require regulatory intercellular contacts with the stroma of hematopoietic organs for their normal proliferation and differentiation [3]. In the same way, stem cells of the bone marrow stroma require adequate intercellular interaction for normal functioning.

LITERATURE CITED

1. I. L. Chertkov and O. A. Gurevich, Probl. Gematol., No. 2, 32 (1979).
2. I. L. Chertkov and A. Ya. Fridenshtein, The Cellular Bases of Hematopoiesis [in Russian], Moscow (1977).
3. I. L. Chertkov (J. L. Chertkov), O. A. Gurevich (O. A. Gurevitch), and G. A. Udalov, Exp. Hematol., 8, 770 (1980).
4. T. M. Dexter, T. A. Allen, and L. G. Lajtha, J. Cell. Physiol., 91, 335 (1977).
5. M. Lanotte, S. Schor, and T. M. Dexter, J. Cell. Physiol., 106, 269 (1981).

INDUCING ACTION OF HYDROCORTISONE ON MITOTIC RHYTHM IN THE CORNEAL AND ESOPHAGEAL EPITHELIUM IN RATS

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KEY WORDS: hydrocortisone; mitosis; corneal epithelium; esophagus.

Glucocorticoid hormones are among the leading factors responsible for adaptation of the body to changing or extremal environmental conditions. Since a persistent increase in the blood levels of adrenocortical hormones is observed in stress, elucidation of the principles governing cell multiplication under conditions of hypercorticism is an urgent problem. Various workers have found a decrease in the number of mitoses and index of [^{3}H]thymidine-labeled nuclei [15, 16] and lengthening of the periods of the mitotic cycle in animals receiving cortisone or hydrocortisone [1, 4]. As a result, glucocorticoid hormones have come to be regarded as inhibitors of cell proliferation [5]. However, this view of the effect of glucocorticoids on proliferation is opposed to their basic (adaptive) role. Inhibition of cell proliferation during prolonged exposure to a stressor, given the more rapid differentiation and death of cells taking place under conditions of hypercorticism, must inevitably lead to a reduction in the number of cells in the tissues and reduction or loss of function. In reality, this does not happen. It must be assumed that the conclusion regarding the inhibitory properties of glucocorticoids is determined by the character of the techniques used in the investigations cited above and many others. First, most studies of cell proliferation during glucocorticoid administration have been undertaken at a particular time of day. We know, however, that the sensitivity of tissues to biologically active substances, including hormones, can vary considerably depending on the time of day when the procedure is carried out [12]. With regard to glucocorticoids, it has been shown that a single injection of dexamethasone can lead to a fall in the mitotic index, can induce synchronization of cell division, or can establish a 48-h period of the mitotic rhythm instead of a 24-h period, depending on the time of injection — in the morning, afternoon, or evening [13]. Second, a transient increase in the corticosteroid level may lead to basically different results from those of prolonged hydrocortisone. If mitotic activity is studied simultaneously in several tissues of rats receiving single or repeated injections of hydrocortisone, it is found to be reduced in animals receiving a single injection of the hormone. In rats receiving injections of hydrocortisone for 1 week before sacrifice, the intensity of cell proliferation is increased. The 24-h pool of DNA-synthesizing cells also is increased [3, 8]. Structure of the mitotic rhythm during intensified cell proliferation induced by hydrocortisone has not yet been investigated. Since circadian rhythms of physiological processes at different levels play an important role in adaptation to the external environment [11], it was decided to study the structure of the mitotic rhythm in the surface epithelium of rats against the background of a prolonged rise in the glucocorticoid hormone level in animals.

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