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Human peripheral blood T lymphocyte subpopulations isolated on the basis of their affinity for sheep red blood cells differ in angiogenesis-inducing capability

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SUMMARY

Human peripheral blood lymphocytes injected intradermally into X-ray immunosuppressed mice were tested for angiogenesis-inducing capability. Both T and B lymphocytes evoked angiogenesis of the same intensity. The total T cell population was fractionated into three subpopulations on the basis of their different affinities for sheep red blood cells (SRBC). Cells belonging to the subpopulation of T lymphocytes displaying moderate affinity for SRBC induced angiogenesis of the highest intensity, higher than that induced by cells of the total T lymphocyte population. However, lymphocytes both with the highest and with moderate affinity for SRBC, mixed together, evoked angiogenesis no different from that evoked by cells of the total T lymphocyte population, suggesting that inhibitory interactions occur among T cells.

INTRODUCTION

Lymphocyte-induced angiogenesis (LIA) has been described as a very rapid and sensitive test for measuring cell-mediated immunity *in vivo* (Sidky & Auerbach, 1975). In this test, an intradermal injection of lymphoid cells into an allo- or semiallogeneic recipient mouse evokes a local graft-versus-host (GVH) reaction, resulting in new blood vessel formation at the injection site. The number of newly formed blood vessels corresponds to the number, origin and immunocompetence of the injected cells (Sidky & Auerbach, 1975; Kamiński, Kamińska, & Majewski, 1978; Auerbach & Sidky, 1979).

In the present study, we have attempted to enlarge the scope of this model and we have tested human lymphocytes on X-ray immunosuppressed mice.

The GVH reaction is evoked mainly by T lymphocytes (Grebe & Streilein, 1976). Human T lymphocytes are shown to possess receptors for sheep red blood cells (SRBC) (cf. Lee, 1977). When incubated together, the SRBC adhere to the surface of a single T lymphocyte and spontaneously form a rosette. A very short incubation time results in rosette formation with only some of the T cells, whereas a longer incubation time permits binding by nearly all T cells. Thus, on the basis of different incubation times it is possible to obtain subpopulations of T lymphocytes with different affinities for SRBC (Nowaczyk, Skopińska & Rdzonek, 1978b). The aim of the present study was to test such T-cell subpopulations for their angiogenesis-inducing capability.

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MATERIALS AND METHODS

Preparation of mononuclear cell suspensions. Samples of defibrinated human blood from healthy donors were obtained from the Blood Transfusion Centre in Warsaw. Mononuclear cells were isolated by the method of Böyum (1968) as modified by Nowaczyk, Skopińska & Rdzonek (1978a), and consisted of 92–97% lymphocytes and 3–8% monocytes as identified by myeloperoxidase staining.

Isolation of T lymphocytes and T lymphocyte subpopulations. The isolation procedure of various T cell subpopulations was performed as described by Nowaczyk *et al.* (1978b). Briefly, samples of 0·2 ml of lymphocyte suspension in PBS ($1{\cdot}0 \times 10^7$ cell per ml), 0·5 ml of PBS supplemented with 10% AB serum (heat-inactivated and SRBC-absorbed) and 0·25 ml of SRBC in PBS ($8{\cdot}0 \times 10^8$ cells per ml) were mixed and centrifuged at 200 ***g*** for 5 min. The pellet was gently resuspended, layered over 1 ml of Ficoll–Uropoline mixture (room temperature) and centrifuged for 11 min at 1,000 ***g***. Rosette-forming cells were collected, washed and subjected to haemolysis, washed and suspended again in PBS. The process of rosette formation was repeated with cells remaining at the Ficoll–medium interface. On the basis of different affinity for SRBC the following subpopulations of T cells were obtained:

(a) A subpopulation displaying the highest affinity for SRBC, forming rosettes immediately. This fraction, designated ARFC, constitutes $26{\cdot}0 \pm 1{\cdot}2\%$ of the total mononuclear cell suspension (Nowaczyk *et al.*, 1978b).
(b) A subpopulation displaying moderate affinity for SRBC, forming rosettes during incubation in the cold (4°C), after removal of the ARFC subpopulation. This fraction, designated C1RFC, constitutes about 22% of the total mononuclear cell suspension (Nowaczyk *et al.*, 1978b).
(c) A subpopulation displaying low affinity for SRBC, forming rosettes during a 2-hr incubation in the cold, after removal of the ARFC and C1RFC subpopulations. This fraction, designated C2RFC, constitutes about 20% of the total mononuclear cell suspension (Nowaczyk *et al.*, 1978b).
(d) A subpopulation containing both ARFC and C1RFC cells, obtained after a 1-hr incubation of the total lymphocyte with SRBC.
(e) A subpopulation of total T lymphocytes, obtained after a 2-hr incubation with SRBC.

Isolation of B lymphocytes. B lymphocytes, containing a major fraction of the non-T-cell population, were also isolated for testing in control experiments. The cells were isolated on the basis of their ability to form spontaneous rosettes with mouse red blood cells as described by Gupta, Good & Siegal (1976).

Lymphocyte-induced angiogenesis assay. Swiss male mice, 8–10 weeks old, were X-ray irradiated with a total-body dose of 700 rad (7 Gy; 35 rad/min in air) 2 hr before the assay. The mice were then injected intradermally with tested cells suspended in PBS (0·1 ml of inoculum per injection). Trypan blue was added to the tested inocula in order to facilitate the location of the injection site later on. After 3 days, recipient mice were killed by ether, their skins carefully separated from underlying muscles, and the newly formed blood vessels directed to the injection site were counted according to the criteria proposed by Sidky & Auerbach (1975).

Cell dose-dependence of angiogenesis. A group of mice was injected with viable, unfractionated lymphocytes. Each mouse was given $0{\cdot}5$, $1{\cdot}0$ and $2{\cdot}0 \times 10^6$ cells of the same donor per single injection to find out whether angiogenesis intensity increases in a cell dose-dependent fashion.

Angiogenesis-inducing capability of lymphocytes from different healthy donors. Viable, unfractionated lymphocytes from 15 healthy donors were injected with a dose of $1{\cdot}0 \times 10^6$ cells per injection. Angiogenesis intensity scores were then evaluated by one-way analysis of variance to find out whether the angiogenesis-inducing ability of lymphocytes from different healthy individuals differs.

Angiogenesis-inducing ability of lymphocyte subpopulations. Unfractionated lymphocytes as well as ARFC and C1RFC from the panel of six donors were obtained and tested in such a way that each mouse recipient was injected with all inocula originating from a single donor. The differences

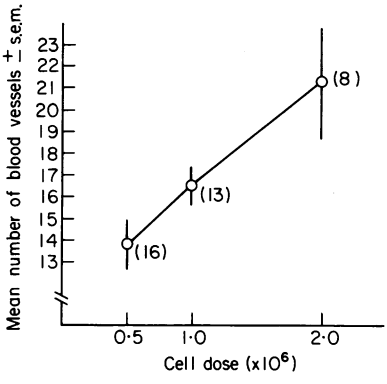

Fig. 1. Angiogenesis dependence on the cell dose injected. Mean numbers of newly formed blood vessels ±s.e. observed on the 3rd day after intradermal injection of peripheral blood lymphocytes into 700-rad-irradiated Swiss mice. No. of injections in parentheses.

between the tested cell subpopulations were calculated excluding the differences between the individuals and mouse recipients. In another series of experiments, unfractionated, as well as T and T cell subpopulations and B population cells and, for control, human erythrocytes, were injected and tested for their angiogenesis-inducing capability.

RESULTS

The angiogenesis dependence on the cell dose was linear within the range of cell doses used (Fig. 1). Fig. 2 shows the angiogenesis-inducing ability of lymphocytes from different healthy donors. The one-way analysis of variance showed no significant differences at $P<0{\cdot}05$ within the tested population. The sampled population mean ±s.e. was $16{\cdot}16\pm0{\cdot}33$ blood vessels per injection site.

The results of the experiment testing T cell subpopulations from the same donor on the same mouse recipient revealed neither significant differences between the donors nor different reactivity of mice at $P<0{\cdot}05$, the latter being greater than the former, however. One million cells from six healthy donors evoked angiogenesis as follows (espressed as the mean number of newly formed blood vessels per single injection site ±s.e.): unfractionated cells $=15{\cdot}28\pm0{\cdot}90$, C1RFC $=19{\cdot}45\pm1{\cdot}62$, ARFC $=18{\cdot}15\pm1{\cdot}99$. The coefficient of variation was greatest within the ARFC population, and lowest within the unfractionated population.

Table 1 shows the pooled results obtained with all of the cells tested.

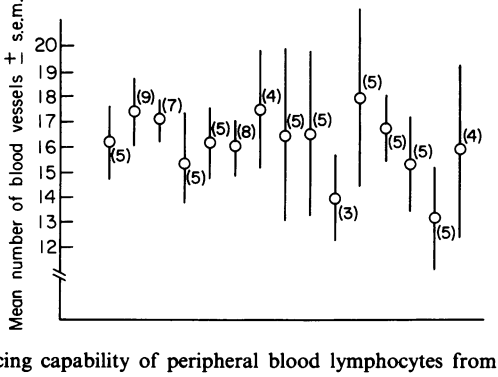

Fig. 2. Angiogenesis-inducing capability of peripheral blood lymphocytes from 15 healthy donors. Mean numbers of newly formed blood vessels ±s.e. observed on the 3rd day after intradermal injection of $1{\cdot}0\times10^6$ cells into 700-rad-irradiated Swiss mice. No. of injections in parentheses.

Table 1. Angiogenesis-inducing capability of human peripheral blood lymphocytes, expressed as the mean number ±s.e. of newly formed blood vessels observed at the injection site on the 3rd day after intradermal administration of $1{\cdot}0 \times 10^6$ viable cells

No. of group	Cells injected	Mean no. of blood vessels at the injection site ±s.e.	Different from group no.†
1	Unfractionated lymphocytes	15·19±0·93 (21)*	2, 3, 6, 8, 9
2	T lymphocytes (ARFC)	18·59±0·93 (32)	1, 5, 8, 9
3	T lymphocytes (C1RFC)	20·36±1·77 (11)	1, 4, 5, 7, 8
4	T lymphocytes (C2RFC)	17·08±1·23 (12)	3, 8, 9
5	T lymphocytes (ARFC+C1RFC)	16·25±0·66 (32)	2, 3, 8, 9
6	B lymphocytes	18·26±1·36 (15)	1, 8, 9
7	Total T lymphocytes	16·88±1·07 (16)	3, 8, 9
8	Human erythrocytes	6·87±0·44 (32)	All
9	Unfractionated lymphocytes ($2{\cdot}0 \times 10^6$ cells)	21·37±1·66 (16)	1, 2, 4, 5, 6, 7, 8

* Number of injection in parentheses.

† The results were tested by one-way analysis of variance and further analysis included Duncan's New Multiple Range test at $P < 0{\cdot}05$.

DISCUSSION

The results show that human peripheral blood lymphocytes are capable of inducing angiogenesis in a xenogeneic host. The number of newly formed blood vessels is correlated linearly with the number of cells injected, at least within the cell dose range used in the present study. The number of blood vessels induced by lymphocytes of each of 15 healthy donors does not differ significantly from the sample mean. Such distribution of angiogenesis-inducing capability within the healthy donors makes it reasonable to investigate whether this ability varies in different conditions.

The ability to evoke angiogenesis is expressed equally by T and B cells. The nature of the lymphocyte-induced angiogenesis mediator is not known, and it may be speculated that the mediator is a kind of a lymphokine; it has been shown that a variety of lymphokines may be produced by human T and B cells (cf. Rosenstreich & Wahl, 1979). In allogeneic systems in mice, B cells, however, are incapable of evoking angiogenesis and lymphocyte-induced angiogenesis in such a system represents a typical GVH reaction induced by T cells only (Sidky & Auerbach, 1975).

Subpopulations of human T lymphocytes obtained in the present study on the basis of their different affinity for SRBC differ in angiogenesis-inducing ability. The highest angiogenic potency is displayed by the subpopulation of T lymphocytes forming rosettes within 1 hr of incubation time (C1RFC)—higher than that displayed by the total T lymphocyte population. Angiogenesis evoked by $1{\cdot}0 \times 10^6$ C1RFC reaches values not significantly differing from angiogenesis evoked by $2{\cdot}0 \times 10^6$ unfractioned cells. Immediate (active) rosette=forming cells (ARFC) also evoke angiogenesis higher than unfractionated lymphocytes but when mixed with C1RFC, an inhibitory interaction occurs and the intensity of angiogenesis does not differ from that evoked by unfractionated cells.

The lymphocyte-induced angiogenesis assay is a rapid, sensitive and inexpensive assay for cell-mediated immunity (Sidky & Auerbach, 1975; Kamiński *et al.*, 1980) and seems to be devoid of the reported disadvantages of the normal lymphocyte transfer test (Gaffney 1978), since those

injections which are not technically good can easily be observed at the inner surface of the skin and excluded from further consideration. The assay would therefore seem to be suitable for testing human lymphocytes in terms of their immunocompetence and/or their angiogenesis-inducing capacity.

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