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Differences in GM-CSF Production from Mouse Peripheral Blood and Spleen Cells Stimulated by Different Lectins

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The capacity of mouse peripheral blood and spleen mononuclear cells to produce GM-CSF or CSA, in response to the stimulation by different mitogens (PHA, PWM and ConA) was studied. Each different kind conditioned medium was tested on target bone marrow from BALB/C mice.

A significant decrease in the number of CFU-GM, was observed using peripheral blood conditioned medium stimulated by PHA or ConA in comparison with spleen conditioned medium in response to identical mitogens. When PWM is used as source of GM-CSF, significant differences between spleen and blood conditioned media were not observed. The possible significance of these findings is discussed.

In vitro colony formation from normal progenitor hemopoietic cells is completely dependent of the presence of adequate concentration of some specific stimulating factors (CSF), also denominated colony-stimulating activity (CSA), in the culture medium. Hemopoietic colony formation therefore permits simultaneously two types of applications: 1) Its provides a method of enumerating codified hemopoietic progenitor cells, and 2) It is a good method for assaying specific growth stimulating factors (CSF or CSA) operating on target hematologic progenitors.

For the CFU-GM (granulocytic-macrophagic colony forming units) production, adequate concentrations in the culture medium of granulocytic-macrophagic colony stimulating factor (GM-CSF) are necessary. This factor can be isolated from salivary gland, lung, thymus, kidney and spleen (8). Tissues effectives in production of large amounts of GM-CSF *in vitro* are lung, heart bone stromal tissue (3, 6, 10). The most commonly used methods for producing high concentrations of GM-CSF are: pregnant uterus and embryo extracts (2), lymphocyte con-

ditioned medium (1), mouse lung conditioned medium (9), mouse L-cell conditioned medium (6), supernatant of one-way mixed-lymphocyte-reaction (MLR) and from spleen cells of animals with graft-versus-host disease (4).

The purpose of this paper is to study the capacity of mouse peripheral blood and spleen cells for producing GM-CSF in response to the stimulation by different mitogens.

Materials and Methods

Mice. Mice used were 45 weeks old BALB/C from the London Hospital Medical College and bred in our laboratory.

Conditioned medium. Peripheral blood from 10 animals was collected from retroorbital sinus with heparinized sterile capillar. The spleen from 5 animals was removed aseptically, minced with scissors and gently pressed through a mesh screen to produce a single cells suspension. Both peripheral blood and spleen cells suspensions were layered on a Ficoll-Hypaque gradient and centrifugated at 800 *g* at 20° C for 10 minutes. The mononuclear layer was collected and washed three times with McCoy 5A medium (Gibco). The cell concentration was adjusted to 10^6 cells/ml in Mc Coy 5A medium supplemented with 20 % foetal calf serum, antibiotics and L-glutamine. Phytohemagglutinin (PHA), Pockweed mitogen (PWM) and Concanavalin A (ConA) at 0.1 mg/ml (final concentration) were added to 10 ml of the above described cells suspensions respectively, to prepare the different conditioned medium. After seven days of incubation at 37° C in fully humidfied atmosphere of 7.5 % CO_2 in air, the tubes were centrifuged at 3,000 *g* at 20° C for 10 minutes. The supernatant was collected and fractionated in aliquots and stored at —20° C until use.

CFU-GM assay. Bone marrow cells were collected by washing the femur of the animals with 2 ml of McCoy 5A medium and layered on Ficoll-Hypaque gradient and centrifugated at 800 *g* at 20° C for 10 minutes. The mononuclear layer was collected and washed three times with culture medium. The cell concentration was finally adjusted to 2×10^5 cells/ml in 0.5 % agar-medium (Mc Coy 5A enriched with foetal calf serum) at 37° C and plated in 1 ml aliquots on 35 mm Petri dishes. 100 microliters of conditioned medium obtained from both peripheral blood and spleen cells stimulated by the above described different mitogens, were dispensed in duplicated plates as source of GM-CSF. After incubation at 37° C in a fully humidified atmosphere of 7.5 % CO_2 in air for 14 days, granulocytic-macrophagic colonies (aggregates of more than 40 cells) were scored in a inverted microscope (Olympus CK Japan). For the identification of the colonies, the agar dishes were dessicated and stained as previously described by TORRES *et al.* (12).

Statistical significance. Statistical significance of the experiments was assessed by a paired Student's t-test.

Results

The number of CFU obtained with conditioned medium from spleen and peripheral blood cells stimulated with different mitogens (PHA, PWM and ConA) are shown in table I.

It is observed a significant increase in the number of CFU-GM when spleen instead peripheral blood cells-conditioned medium were obtained with stimulation by PHA or ConA. This difference was not observed when PWM was used as promoting of GM-CFS by both spleen and peripheral blood cells. On the other hand, there are not significant differences in the CFU-GM growth when spleen cells

Table I. *Differences in CFU-GM growth in relation with the type of mitogen-induced conditioned medium used as source of C.S.A.* Result represent the arithmetic mean ± S.E.M. of 5 experiments. PHA = Phytohemagglutinin; PWM = Pockween mitogen; CON-A = Concanavalin A.

Mitogen (0.1 mg/ml)	Conditioned medium		
	Peripheral blood	Spleen	p
PHA	21.0 ± 2.40	35.1 ± 3.26	<0.001
PWM	41.4 ± 4.06	39.8 ± 3.66	N.S.
CON-A	26.6 ± 4.06	39.0 ± 3.15	<0.01

N.S. = $p > 0.05$.

are used as source of different conditioned medium, in response to the three studied lectins. However, it can be observed a significant decrease in the CFU-GM growth, when is considered conditioned medium from peripheral blood in response to PHA and ConA in comparison with PWM.

Discussion

Table I shows that GM-CSF production in response to PHA and ConA, is significantly higher when spleen cells are used instead of peripheral blood cells as source of conditioned medium. However, there were no significant differences in CFU-GM growth whether we added PWM-peripheral blood-CSF or PWM-spleen cells-CSF

It is well known that PHA and ConA stimulate selectively T-cells, whilts PWM stimulate both T and B lymphocytes (5). In this sense, lymphocytes seem to have a crucial role in the CSF production, since thymectomy reduces the number of CFU-S in mouse bone marrow (14) and the simultaneous injection of thymocytes and bone marrow cells improve the growth of CFU-S in the spleen (11).

Lymphocytes are important factories of different lymphokines and it has been suggested that lymphocytes themselves are able to produce CSA (13). However, it has been also suggested that mononuclear phagocytes may be stimulated by a lymphokine to release CSA, having the lymphocytes an indirect action (4). Thus, our results could be explained by the different action of mitogens on several lymphocytes subpopulations, although it can be also possible that the differences in CSA production observed, could be due to the different effect of these lectins acting directly on the macrophages, since it has been stablished that PHA, ConA and PWM have a different effect on the monokine release by macrophages (7).

Our results do not permit to clarify if the differences in the GM-CSF production are due to a different effect of each mitogen acting on lymphocytes, modificating the lymphokines releasing from these cells, or if these lectins act directly changing the CSF production from mononuclear phagocytes. However, further studies of these cell populations and their interactions are necessary to provide more information about the role of lymphocytes and macrophages on hemopoiesis and its importance in clinical situations.

Resumen

Se estudia la capacidad de las células mononucleares de sangre periférica y de bazo de ratón BALB/C para producir GM-CSF o CSA, en respuesta a la estimulación con diferentes mitógenos (PHA, PWM y ConA). Cada uno de los distintos medios condicionantes obtenidos fueron valorados sobre médulas óseas dianas precedentes de la misma cepa de ratones, para ver la capacidad de inducir el crecimiento de CFU-GM. Se observa un descenso significativo en el número de CFU-GM cuando se utiliza medio condicionante procedente de sangre periférica e inducido con PHA o ConA, en comparación con medios condicionantes procedentes de células mononucleares esplénicas estimuladas por estos mismos mitógenos. Cuando se utiliza PWM en la estimulación de la producción de GM-CSF, no se observan diferencias significaticas entre el crecimiento de CFU-GM indu-

cido por medio condicionante procedente de bazo o de sangre periférica.

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