EFFECTS OF INTRAVENOUS ADMINISTRATION OF ANTIGEN ON LYMPHOCYTE LOCALIZATION AND BLOOD FLOW INTO THE MOUSE SPLEEN

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SUMMARY

The present paper investigates whether the increased lymphocyte localization into the spleen, which occurs early after the i.v. injection of some antigens, is related to an increase in the blood flow to that organ. For this purpose the fractional distribution of cardiac output to the spleen using the ^{86}Rb Cl method, was studied at different intervals after the i.v. administration of agents known to be able to induce strong *trapping*. The results show no correlation between the accumulation of recirculating cells into the spleen and changes in blood flow.

RESUMO

Efeitos da administração de antigénio por via intravenosa sobre a localização linfocitária e a perfusão esplénica do rato

Procura-se esclarecer se a concentração linfocitária que se verifica no baço logo após a administração i.v. de diversos antigénios está ou não relacionada com o aumento de perfusão deste órgão. Usando o ^{86}Rb Cl estudou-se a fracção do débito cardíaco destinada ao baço, após a administração de agentes capazes de provocar uma acentuada concentração do material radioactivo no órgão. Verificou-se não haver correlação entre a acumulação de células no baço e as variações de perfusão sanguínea.

INTRODUCTION

The injection of an antigen can induce an increased localization of recirculating cells in the draining lymphoid organs, which occurs early after administration and affects all cells in transit regardless of their antigen specificity.[1]

This phenomenon is directly related to the nature of the antigen, particulate antigens being more effective than soluble proteins,[2, 3] and can also be induced by adjuvants and inerte particles.[2, 3] It is present in lymphocyte deprived mice and can be transferred by macrophages harvested from the spleen or peritoneal cavity of stimulated donors.[4]

Studies in sheep have clearly demonstrated that the increased localization of cells in the draining lymph nodes following antigen administration was due to an increased entry of cells into the node proportional to an increase in blood flow to that organ.[5, 6]

Early studies on the output on the isolated rat spleen[7] suggested, however that this phenomenon was due to the non-specific retention of circulating lymphocytes rather than increased entry of cells into the stimulated spleen. In order to investigate the mechanisms which lead to the increased in vivo cell localization into the spleen, we studied the fractional distribution of cardiac output (C.O.) to this organ at different time intervals after antigen administration. Our results show a lack of correlation between blood flow and the increased spleen localization of cells.

MATERIALS AND METHODS

Mice — C_3H/An inbred mice obtained from Cumberland View Farms and Balb/inbred mice from the Instituto Gulbenkian de Oeiras were used. For each experiment mice were matched for age (6-8 weeks) and sex.

In vitro labelling of cells

Lymph node cells were prepared by gentle teasing of the mesenteric, inguinal and brachial nodes in MEM (Gibco, Bio-Cult Ltd. Scotland) containing 10% fetal calf serum (FCS) (Gibco, Bio-Cult, Ltd.).

To label small lymphocytes, cell suspensions were incubated with (^{51}Cr) sodium chromate (Radiochemical Center) at a concentration of 50 μCi/10^8 cells/ml of MEM with 10% FCS at 37 °C for 30 min. After lebelling cells were washed three times in medium alone (MEM). To remove debris and dead cells the cell suspension were passed through a column of glass wool. The number of viable cells, as assessed by Trypan blue (0.1%) exclusion test, was greater than 95%.

Treatment of recipients

Mice were either left untreated or injected i.v. with latex beads (0.1 ml of a 10% suspension), LPS (10μg/mouse) or SRBC (0.2 ml of 10% suspension) at various times before cell transfer or injection of Rb Cl.

Received: 18th November, 1981

Measurement of the Cardiac Output distribution (% CO)

C.O. distribution was measured by a modification of the method originally described by Sapirstein, L. A., (1958), using RbCl. Under light ether anaesthesia an intravenous cannula was inserted into a lateral vein of the mouse under study. A volume of 0.9% saline was injected to ensure patency followed by injection of known volume of ^{86}Rb Cl diluted with 0.9% saline. This was immediately flushed through with an equal volume of 0.9% saline. After 45 s, the animal was killed by injecting saturated K Cl via the same cannula. The tissues of interest were then dissected out and placed in plastic containers for counting, in a gamma spectometer (Packard).

The percentage of the cardiac output (% CO) going to a particular organ was expressed as the total ^{86}Rb activity which was found in that tissue as a percentage of the total ^{86}Rb activity injected as shown in equation 1.

$$\%\,C.O._i = \frac{^{86}\text{Rb counts}}{^{86}\text{Rb injected}} \times 100 \text{ (equation 1)}$$

The specific splenic perfusion was determined as the percent of cardiac output per mg of tissue.

Measurement of the *in vivo* distribution of labelled cells

Injection dose of 10^7 ^{51}Cr labelled cells was given through a lateral tail vein of syngeneic recipients in a 0.2 ml volume. Multiple samples of each injection dose were retained for counting of the administered radioactivity. 24 h after cell tranfer the animals were sacrified and the tissues of interest dissected out and counted in a gamma-spectometer (Packard).

The in vivo distribution of labelled cells within a given tissue was expressed as a percentage of the injected dose of radioactivity.

Statistical Analysis

The significance of differences in means of various groups was assessed using Student's t test. Correlation coefficients (r) were calculated by standard methods.

RESULTS

The distribution of ^{86}Rb Cl activity in normal mice

Using an indicator such as ^{86}Rb Cl, the portion of an intravenous injection dose which is found in a particular organ within 1 min. is a measure of the fraccional distribution of the C.O. which is received by that tissue.[8] The profile of distribution of the cardiac output (Table 1) is in good agreement with that found in previous studies both in mice and other species.[6, 8, 9, 10] As expected, the perfusion per gram of the different lymphoid tissues is substantially less than highly perfused tissues such as the kidney (not shown).

TABLE 1 **BLOOD FLOW: Expressed as a % C.O. in different organs of mice**

	RK	LK	Liv	Lung.	Spleen	MLN
% C.O.	3.29a) ±1.4	3.30 ±1.3	8.62 ±1.7	3.96 ±0.6	0.92 ±0.4	0.32 ±0.1

a) Mean ± S.D. of 132 mice.

Assessment of the symmetry of Rb Cl distribution

If the Rb Cl is adequately mixed with the blood leaving the left ventricle, then it should distribute evenly to organs having a bilaterally symmetrical distribution. Fig. 1 shows the distribution to the two kidneys in the mice studied. The results show that the left and right kidney received an identical amount of Rb Cl in every animal examined.

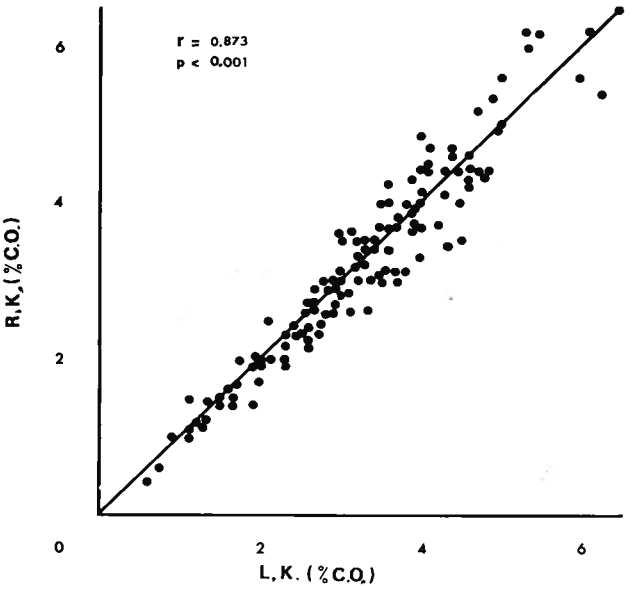

Figura 1: The distribution of ^{86} Rb Cl to the kidneys. The percentage of the injected radioactivity recovered in the left kidney is plotted against the right kidney for each animal. The theoretical line of identity is drawn, and the correlation coefficient and its significance value are shown.

Blood flow in the spleen after antigen administration

In order to investigate whether *lymphocyte trapping* in the spleen could be the result of an increased blood flow we studied the fractional distribution of the C.O. which was received by the spleen at different time intervals after i.v. injection of either LPS (10 μg/mouse), SRBC (0.2 ml of a 10% suspension) or latex beads (0.1 of a 10% suspension) in conditions where these agents induced an increased localization of cells in the spleen (Tables, 2, 3, 4).

In order to interpret the results it must be noted that there is a time lag between the measurement of blood flow which is done immediatly at 1, 6, 24 and 48 hours after antigen arrival, and cell localization which is only assessed 24 h after cell transfer and thus occurs effectively 24, 30 and 48 h after antigen.

Injection of SRBC or latex beads i.v. did not induce significant increases in organ weight, blood flow or specific splenic perfusion within the first 24-48 h after injection of the antigen, i.e. at the time when cell trapping is maximal (Tables 2, 3). Administration of LPS, however, induced a significant increase in organ weight and blood flow to the spleen 1, 24 and 48 h after Ag administration (Table 4). The differences in blood flow observed 24 h and 48 h after LPS injection could be attributed to an increase in organ weight (Table 4) since the splenic perfusion rate (% C.O./mg) was unmodified at times when differences in cell localization were observed (Table 4).

We conclude, that there is no correlation between changes in blood flow and the increased cell localization of recirculating cells after antigen administration.

TABLE 2 **The effects of an i.v. injection of latex beads in cell localization, blood flow and organ weight in the spleen.**

	Untreated	L. B. treated		
		1 h[a]	6 h	24 h
% injected [b] radioactivity	18.3 ± 2.2	21.6 ± 1.2 *	19.7 ± 1.0	20.8 ± 0.7 *
% C.O./mg [c]	0.0079 ± 0.0037	0.0096 ± 0.0038	0.00099 ± 0.0038	0.0081 ± 0.0032
% C.O.	0.92 ± 0.44	1.13 ± 0.41	1.06 ± 0.36	0.90 ± 0.23
Weight (mg) of organ	119 ± 20	123 ± 21	112 ± 13	119 ± 30

a) Time after i.v. injection of 0.1 ml of a 10 % suspension of latex beads.
b) Syngeneic ^{51}Cr labelled cells were injected 1, 6 or 24 hours after latex beads administration and their spleen localization assessed 24 h after transfer.
c) The results shown represent the mean ± S.D. of 6 to 32 mice (for blood flow exp. at least 12 mice/group were used).
* $p \leq 0.01$

TABLE 3 **The effects of an i.v. injection of SRBC in cell localization, blood flow and organ weight in the spleen.**

	Untreated	SRBC			
		1 h[a])	6 h	24 h	48 h
% injected [b] radioactivity	18.3 ± 2.2 [c])	27.8 ± 1.6 **	25.9 ± 2.0 **	25.7 ± 1.6 **	
% C.O./mg	0.0079 ± 0.0037	0.0093 ± 0.0034	0.0077 ± 0.0030	0.0067 ± 0.0019	0.0091 ± 0.0029
% C.O.	0.92 ± 0.44	1.10 ± 0.38	0.92 ± 0.33	0.80 ± 0.25	1.10 ± 0.41
Weight/organ	119 ± 20	119 ± 18	121 ± 19	122 ± 27	122 ± 16

a) Time after i.v. injection of 0.2 ml of a 10 % SRBC suspension.
b) Syngeneic ^{51}Cr labelled cells were injected 1, 6, 24 h after SRBC administration and their spleen localization assessed 24 h after transfer. The results shown represent the mean ± SD of groups of 6-32 mice (for blood flow measurements at least 12 mice/group were used).
** $p \leq 0.001$.

TABLE 4 **The effects of an i.v. injection of LPS in cell localization, blood flow and organ weight in the spleen.**

	Untreated	LPS Treated			
		a)	6 h	24 h	48 h
% injected [b] radioactivity	18.3 ± 2.2 [c])	19.1 ± 2.1	19.6 ± 1.1	25.9 ± 1.5 **	
% C.O./mg	0.0079 ± 0.0037	0.0106 ± 0.0019	0.0062 ± 0.0020	0.0090 ± 0.0036	0.0091 ± 0.0029
% C.O.	0.92 ± 0.44	1.28 ± 0.19 *	0.82 ± 0.26	1.30 ± 0.59 *	1.28 ± 0.20 *
Weight/organ	119 ± 20	122 ± 13	134 ± 17 *	145 ± 29 **	140 ± 20 *

a) Time after i.v. injection of 10 mg of LPS.
b) Syngeneic ^{51}Cr labelled cells were injected 1, 6, 24 h after LPS administration and their spleen localization assessed 24 h after transfer. The results shown represent the mean ± SD of groups of 6-32 mice (for blood flow measurements at least 12 mice/group were used).
* $p \leq 0.01$.
** $p \leq 0.001$

DISCUSSION

Lymphocyte *trapping* is defined as the accumulation of recirculating lymphocytes regardless of their antigen specificity which occurs in a lymphoid organ early after antigen arrival.[1, 2, 3] This phenomenon is well documented for lymph nodes where it was demonstrated to be the result of an increased input of cells proportional to an increased blood flow to the node.[5, 6] It was shown that this early increase in blood flow was specific, i.e., due to an increased rate of perfusion to the node, rather than the result of changes in organ size and vascular neoformation. The present study is, however, the first to investigate whether changes in regional blood flow to the spleen could account for changes in lymphocyte after antigen arrival. To test this hypothesis the splenic blood flow was measured in individual mice at various stages after the i.v. administration of agents known to induce early cell accumulation to that organ. On the basis of the results obtained we conclude that there is no correlation between blood flow and increased cell accumulation in the spleen, after the i.v. injection of either SRBC, latex beads or LPS.

It might be argued that due to the individual variation of flow to the spleen the method employed was not sensive enough to detect small changes in splenic perfusion. It must be noted, however, first that the profile of distribution of the cardiac output and specific splenic perfusion rates are in good agreement with those found in previous studies both in mice and other species.[6, 8, 9, 10] Secondly, 1, 24 and 48 hours after the injection of LPS there were detectable differences in blood flow to the spleen. These differences, were either specific but unrelated to the increased cell localization to the spleen or non specific due to the increase in organ weight, i.e., the specific splenic perfusion was unmodified.

The present results suggest that in the spleen the early accumulation of recirculating cells after antigen arrival is due to a true retention of cells within the organ. This is in agreement with results obtained in *in vitro* experiments performed with the isolated perfused spleen, which have shown that after antigen administration there was a decrease on the output of cells into the spleen perfusate.[7]

In contrast with our observations, studies on the mechanisms of lymphocyte *trapping* into the lymph nodes have clearly shown that the increased cell localization is the result of an increased blood flow to the nodes.[5] The reasons for these discrepancies may lie on different vascular supplies of lymph nodes and spleen. It is however possible that changes in blood flow to the spleen occur exclusively at the level of the white pulp, which would be undetectable as we only study the overall specific perfusion rate. Alternatively, divergent mechanisms of *lymphocyte trapping* can exist for lymph nodes and spleen, which may be relevant to their function, specially if we consider the different nature of the antigens which in physiological circunstances reach these organs.

ACKNOWLEDGMENTS

We thank M. Isabel Simões and M. Luís for excellent secretarial assistance.

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