Acta virol. **26** : 91—97, 1982

ROLE OF T LYMPHOCYTES IN *RICKETTSIA CONORII* INFECTION

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Received June 3, 1981

Summary. — Adoptive transfer of T lymphocytes harvested from spleen of *Rickettsia conorii*-infected DBA/2 mice to intact and cyclophosphamide- (CPA-) pretreated syngeneic mice protected the latter from lethal infection caused by *R. conorii*. Protection from infection was not observed in recipient mice given immune serum or B lymphocytes and macrophages from the spleens of convalescent mice. The protective effect was most pronounced after intravenous transfer of lymphocytes obtained from donor mice on day 14 post infection. Lethal infection was not prevented by transfer of lymphocytes harvested on day 50 p. i., although at this interval the donor mice were still resistant to reinfection with *R. conorii*.

Key words: Rickettsia conorii; T lymphocytes; adoptive transfer; immunity

Introduction

More and more data have accumulated indicating that the protection from rickettsial infection is ensured by a complex of cellular and humoral factors (Wisseman, 1974, 1978; Kazár, 1978; Kokorin and Kabanova, 1979; Kokorin *et al.*, 1980; Vovk *et al.*, 1980).

The immune response is realized by different populations of lymphocytes and macrophages. As to immunity in rickettsial infections, the role of macrophages as effector cells for elimination of rickettsiae has been studied the most intensively (Kishimoto and Walker, 1976; Kishimoto *et al.*, 1977; Kabanova *et al.*, 1978; Nacy and Osterman, 1979; Kokorin and Kabanova, 1979; Kokorin *et al.*, 1980; Vovk *et al.*, 1980). The decisive specific role of lymphocytes in prevention from lethal rickettsial infection was determined by adoptive transfer of immune cells obtained from the spleen or peritoneal exudate to intact recipient animals (Shirai *et al.*, 1976; Kazár *et al.*, 1977; Kabanova *et al.*, 1978; Kokorin and Kabanova, 1979; Kobayashi *et al.*, 1979; Murphy *et al.*, 1979). This protective effect was attributed to T lymphocytes (Shiral *et al.*, 1976; Kokorin and Kabanova, 1979; Kobayashi *et al.*, 1979).

The purpose of our study was to investigate the protective role of spleen cells (T lymphocytes, B lymphocytes, and macrophages), obtained from mice at different stages of infection with *Rickettsia conorii*, by their adoptive transfer to syngeneic recipient mice, and to determine whether resistance of donor mice to reinfection with *R. conorii* would depend on the protective function of lymphocytes.

Materials and Methods

Inbred DBA/2 mice weighing 16-18 g were used troughout. Donor mice were inoculated intraperitoneally (i.p.) with 10^3 EID_{50} of *R. conorii* strain M-1 (grown in chick embryo yolk sacs and stored at −60° C) in 0.5 ml volumes. Recipients of lymphoid cells were either intact mice or mice pretreated with one i. p. dose of cyclophosphamide (CPA/300 mg per kg body weight) 4 hr before injection of spleen cells from donor mice. Intact recipient mice were infected with 10^4 EID_{50} of *R. conorii* 24 hr after intravenous (i.v.) administration of donor spleen cells. Because CPA is known to enhance rickettsial infection (Kazár *et al.*, 1971), CPA-pretreated mice were infected with lower doses (10^1—10^3 EID_{50}) of *R. conorii* at the same interval.

The lymphoid cells were harvested from the spleens of *R. conorii*-infected mice 6, 14, 30 and 40-50 days p.i. by teasing the pulp from the spleen capsule into medium 199 containing 10% foetal calf serum. To remove erythrocytes, the spleen cells were treated with a 0.84% HN_4 Cl aqueous solution. The syngeneic recipients were injected i.v. with 10^8 lymphoid cells in 0.3 ml volumes.

Enriched T- and B-lymphocyte populations of spleen cells were obtained by the method of Kadar *et al.* (1974) as modified according to Khazova (personal communication). To 1.0-1.5 ml spleen cell suspension (containing 10^8 cells per ml medium 199), 0.3 ml of a 10% suspension of sheep erythrocytes sensitized by anti-erythrocyte serum was added. The mixture was centrifuged for 5 min at 500 rev/min and incubated for 30 min at 37° C. The sediment was then thoroughly resupended, layered on a serum gradient and centrifuged for 5-7 min at 700-800 rev/min. The resulting sediment contained B-lymphocyte and the supernatant T-lymphocyte enriched cell populations, respectively.

To inactivate T lymphocytes, the spleen cells were incubated with anti-T globulin (ATG) prepared from anti-lymphocyte globulin (ALG) batch No. 443, kindly supplied by Dr. N. A. Kraskina, Institute of Epidemiology and Microbiology, Moscow.

ATG was prepared according to Kraskina (1976) by absorption of ALG with mouse liver homogenate (3 times), with mouse erythrocytes (3 times), and with spleen cells from the so-called B mice (twice), followed by precipitation of globulin with ammonium sulphate. The spleen cells were incubated with ATG and complement, or with complement alone, for 30 min at 37° C.

In some experiments, mice were bled on days 14 and 21 p.i. (and also 21 days after reinfection); sera for mice of the same group were pooled and examined in the complement-fixation (CF) reaction with *R. conorii* antigen.

Results

As shown in Table 1, CPA-pretreatment of mice increased markedly their susceptibility to infection with *R. conorii*. Based on these results, most experiments were carried out in CPA-pretreated mice infected with 10^2—10^3 EID_{50} of *R. conorii*.

The spleen cells harvested from syngeneic immune donors on day 14 p. i. with *R. conorii*, but not from intact (control) mice, protected both intact and CPA-pretreated recipients from lethal *R. conorii* infection (Table 2). The course of rickettsial infection in CPA-pretreated mice differed also from that in intact, CPA-untreated mice. Intact recipient mice given spleen cells from immune donors never died, they even did not develop any signs of disease.

Table 1. The effect of CPA on R. conorii infection in DBA/2 mice

Mice	Inoculum (EID_{50}) 10_4	10^3	10^2	10^1	1
Intact	0/15	15/20	10/10	10/10	Not tested
CPA-pretreated	0/33	0/21	0/13	3/8	9/10

Numerator: No. of surviving mice; denominator: No. of inoculated mice.

On the other hand, spleen cells from immune donors protected CPA-pretreated mice from death, but not from development of rickettsial infection (the mice were flaccid and showed lower mobility and ruffled hair). In peritoneal smears from CPA-pretreated mice, up to 50% of cells contained rickettsiae on day 4 p. i. with a clearance of rickettsiae on days 7-8 p. i. The CF antibody titres in this group of mice always exceeded those in intact mice (Table 3).

To determine the role of T lymphocytes in protection from *R. conorii* infection, the spleen cells from immune donors were treated with ATG known to inactivate T cells. *R. conorii*-infected CPA-pretreated recipient mice were divided into three groups: (*a*) those given untreated spleen cells from immune donors; (*b*) those given the same cells but treated with complement; and (*c*) those given the same cells after treatment with complement and ATG (Table 4). About 2-3 times more mice survived in the groups *a* and *b* than in group *c*. A marked difference was also found in the rickettsia-containing cell counts in the peritoneal smears from recipient mice given ATG-treated or ATG-utntreated immune spleen cells. The proportion of rickettsia-infected cells and the amount of rickettsiae per infected cell was higher at all intervals tested in mice given immune spleen cells deprived of T lymphocytes (Table 5).

These data offered evidence of the role of T lymphocytes in protection from *R. conorii* infection conferred on recipient mice by spleen cells from immune syngeneic donors. This conclusion was confirmed by further expe-

Table 2. Protection of mice from R. conorii infection conferred by spleen cells from immune and control donors

Recipient mice	Donor of spleen cells	Proportion* of mice infected with *R. conorii* doses of 10^4 EID_{50}		10^3 EID_{50}	
Intact	Immune	9/10	90%	—	
	Control	1/8	13%	—	
	None	0/15		—	
CPA-pretreated	Immune	8/13	63%	37/40	91.5%
	Control	1/12	9%	1/23	5.0%
	None	0/33		0/21	0

* Numerator: No. of surviving mice; denominator: No. of inculated mice.
— = Not tested.

Table 3. CF antibody titres in different groups of mice 21 days p. i. with 10^3 EID_{50} of R. conorii

Group of mice	Antibody titres in experiments 1	2	3	4	5
CPA-pretreated recipients*	160	640	1280	640	160
Intact recipients*	20	20	—	—	20
Infected once	—	160	80	—	80
Reinfected after 14 days	40	80	—	—	40
Reinfected after 35 days	40	20	—	—	40

* Recipients of spleen cells from immune donor mice.
— Not done.

riments aimed at the adoptive transfer of immunity by populations of immune spleen cells enriched with T or B lymphocytes and macrophages. Whereas 90—100% of animals survived in the groups of 8-9 mice injected with the whole population of immune spleen cells or with a T-lymphocyte enriched population, as many as 75% (6 of 8) of mice given B-lymphocyte- and macrophage-enriched population of immune spleen cells died up to the 7th-10th day p. i. Protection from lethal *R. conorii* infection did not occur in recipient mice given a population of immune spleen cells enriched with B lymphocytes and macrophages, like in mice given immune serum with a CF antibody titre of 640, collected on days 14-21 p. i. with *R. conorii*.

The protection of mice was the most pronounced (90—100%) when the spleen cells were harvested on day 14 p. i.; with spleen cells collected on days 6 and 30 p. i., the protection was lower (up to 20 % and 35%), collected on days 6 and 30 p. i., respectively, and no protection was conferred by spleen cells harvested from donor mice 50 days p. i. At the same time, i. e. on days 14, 30 and 50 after primary infection, the mice were resistant to reinfection with *R. conorii*.

Discussion

Our results offered evidence of the role of T lymphocytes in protection of mice from death caused by *R. conorii* infection, and under certain circumstances also in protection from developing rickettsial infection. However, no protection of mice from the lethal *R. conorii* infection was observed upon adoptive transfer of B lymphocytes and macrophages from immune donors or by administration of immune serum.

Table 4. The effect of ATG on survival of mice given 10^8 spleen cells from immune donors

Spleen cells from immune donors	Proportion* of mice infected with *R. conorii* doses of 10^4 EID_{50}		10^3 EID_{50}	
Untreated (*a*)	8/13	63%	24/27	89%
Treated with complement (*b*)	11/16	67%	14/14	100%
Treated with ATG and complement (*c*)	3/17	18%	8/16	50%

* Numerator: No. of surviving mice; denominator: No. of inoculated mice.

Table 5. The effect of ATG on the proportion of R. conorii-infected peritoneal cells in recipient mice given spleen cells from immune donors

Spleen cells from immune donors	% of rickettsia-containing peritoneal cells on days p.i.		
	4	5	6
ATG-treated	84.7	87.0	60.0
ATG-untreated	50.0	14.5	5.5

So far, the protective role of lymphocytes has been demonstrated in animal infections with *Rickettsia tsutsugamushi* (Shirai *et al.*, 1976; Catanzaro *et al.*, 1977; Kobayashi *et al.*, 1979), *Coxiella burnetii* (Kazár *et al.*, 1977), *Rickettsia typhi* (Murphy *et al.*, 1979), and *R. conorii* (Kabanova *et al.*, 1978; Kokorin and Kabanova, 1979). As follows from studies on *R. tsutsugamushi* and *R. conorii* infections, T lymphocytes are responsible for adoptive transfer of immunity.

T lymphocytes are known to produce lymphokines — mediators enabling the cooperation of T lymphocytes with other cells of lymphoid and haemopoietic systems by influencing their functional activities. Significance of lymhopkines was also demonstrated in rickettsial infections, namely their ability to activate macrophages for an increased rickettsial killing (Hinrichs and Jerrells, 1976; Nacy and Osterman, 1979). In adoptive immunity, transfers of the macrophages alone did not prevent recipient animals from rickettsial infection (Kazár *et al.*, 1977; Kokorin and Kabanova, 1979).

The protective function of lymphocytes from immune donors differed depending on the time of their harvest in the course of the infection. In our case, the functional activity of spleen lymphocytes was the most pronounced on day 14 p. i., but it was absent on day 50 p. i. irrespective of the persistence of resistance to *R. conorii* reinfection at the latter interval. The loss of protective capacity of spleen lymphocytes might be caused either by the appearance in the spleen of other functional subpopulations of lymphoid cells (T-suppressor cells) or by disappearance from the spleen of functionally active T lymphocytes. Such a migration of active lymphocytes from the spleen was confirmed indirectly by Catanzaro *et al.* (1977), who found a decrease in the protective function of spleen lymphocytes obtained from mice injected i. p. with mineral oil, whereas protective activity of lymphocytes from the peritoneal exudate was well preserved.

As follows from previous studies (Kokorin and Kabanova, 1979; Mishkarova *et al.*, 1980), activity of at least three factors, i. e. sensitized T lymphocytes, activated macrophages, and specific antibodies, appeared on day 14 p. i. with *R. conorii*. At later intervals (on days 40-50 p. i.), decrease in antibody titres, in protective function of spleen lymphocytes and in inhibition of macrophage migration reaction was accompanied by preservation of a high metabolic activity of macrophages (Kokorin *et al.*, 1980) and by specific resistance of animals to *R. conorii* reinfection. According to Catanzaro *et al.* (1977), the protective function of lymphocytes from the peritoneal exudate, which

differ qualitatively from the spleen lymphocytes, is still maintained at later intervals.

The change in the activity of indicators of immunity and parallel preservation of resistance of animals to infection suggest the phase character of immunological activity of different populations of lymphoid cells. It obviously indicates an absence of a full correlation between intensity of individual cellular and humoral reactions, and reaction of the host organism to reinfection. In rickettsial infections of animals, the most long-lasting is an increased activity of the spleen and peritoneal macrophages (Kokorin *et al.*, 1980; Kekcheeva *et al.*, 1981), and of lymphocytes from the peritoneal exudate (Catanzaro *et al.*, 1977).

An increased metabolic activity of macrophages could be connected with different factors, including mediators produced by T lymphocytes which may de-repress some enzymatic macrophage systems preventing the multiplication, and stimulating the killing, of intracellular rickettsiae.

Finally, it should be mentioned that different populations of lymphoid cells take part in protection from rickettsial infection: T lymphocytes from the spleen and peritoneal exudate, producing lymphokines and warranting protection from lethal infection in adoptive immunity transfers; B lymphocytes producing specific antibodies; and macrophages as effector cells in the process of infection.

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