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# *COXIELLA BURNETII* ANTIGENS MAY INDUCE RESISTANCE TO TUMOUR GROWTH

N. K. TOKAREVICH, A. M. MALIGIN, T. A. KRAMSKAYA, O. N. POGODINA

Pasteur Institute of Epidemiology and Microbiology, 197 101 Leningrad,
Institute of Cytology of the Academy of Medical Sciences of the U.S.S.R.,
194 064 Leningrad, and Institute of Experimental Medicine,
Academy of Medical Sciences, 198 020 Leningrad, U.S.S.R.

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*Summary.* — *Coxiella burnetii* antigens stimulate the defence against growth of hepatoma 22a cells. The antigen-stimulated mice survived longer, they considerably later developed palpable tumours and showed a retarded tumour growth. The enhanced resistance to tumour growth may be explained by at least 2 interrelated phenomena; namely by the induction of interferon-like activity and an increased NK cell activity.

*Key words*: *Coxiella burnetii*; *tumour resistance*; *natural killer cells*; *interferon-like activity*

## *Introduction*

From a great many immunomodulators described recently, several are of bacterial origin, *C. burnetii* antigens being no exception (Brezina *et al.*, 1968; Kazár and Schramek, 1979; 1984). It seemed reasonable, in this context, to verify whether the *C. burnetii* antigens would stimulate the defence against hepatoma 22a (MG 22a) cells as these had not been yet used for such investigation.

## *Materials and Methods*

*Antigen administration.* C3HA mice weighing 14 ± 1 g were coming from the breed "Rapalovo" (Academy of Medical Sciences, U.S.S.R.). They were given the corpuscular *C. burnetii* antigen from phase 1 cells (from *Apodemus flavicollis* strain Lugo, passage No. 3) prepared in hatched embryos. Formalin-inactivated *C. burnetii* cells were purified by differential centrifugation and ether treatment. The antigen content was tested by indirect haemagglutination using immunoglobulin coated erythrocytes (Tokarevich *et al.*, 1981); the antigen was dissolved in phosphate buffered saline (PBS) pH 7.4 at a concentration of 200 μg/ml and inoculated intraperitoneally in a volume of 0.5 ml.

*Interferon-like activity* was detected in the sera of antigen recipients with the micromethod described by Pavlushina and Orlova (1981) using $L_{929}$ cells inoculated with encephalomyocarditis virus. The IFN-like activity was expressed in $\log_2$ units representing reciprocals of the virus-inhibiting serum dilution. The serum from mice infected with the Newcastle disease virus, an interferon (IFN) inducer, was used as reference IFN standard.

*Cytotoxic test.* At early intervals after antigen stimulation the mice were sacrificed and splenocyte suspension were prepared removing the erythrocytes by haemolytic shock. The natural

killer cell activity was determined as described by Hashimoto and Sudo (1971). Established line of human erythroblastosis cells was used as target (K-562 cells). The cells were cultured in RPMI-1640 medium supplemented with glutamine, 80 μg/ml gentamycine (Pharmachim, Bulgaria) and 10% foetal calf serum (FCS). They were labelled by adding of 110 kBq $^3$H-uridine (specific activity of 1.85 TBq/mmol, Isotop, U.S.S.R) for 2 hr at 37°C. The rest of the label was removed by three -fold washing and low speed centrifugation.

*The cytotoxic test* was carried out in the medium RPMI-1640 supplemented with 2 mmol/l glutamine, 10% FCS, 80 μg/ml gentamycine and 20 mmol/l HEPES pH 7.3 (Flow Laboratories, U.K.). The test was performed in domestic microplates (Medpolymer, Leningrad). Each wall was given 0.1 ml of nonfractionated splenocyte suspension ($10^6$ cells/ml) and 0.1 ml of the labelled K-562 cells ($10^5$ cells/ml). Control wells did not receive the splenocytes. Each sample was checked 3 times. The mixtures were incubated in $CO_2$ atmosphere for 18 hr at 37°C. The content of the wells was then transferred to paper filters, washed with physiological saline and 5% trichloroacetic acid and ethanol. The radioactivity was measured in Mark-2 scintillation counter (Nuclear, Chicago, U.S.A.). The cytotoxic index was determined according to the formula:

$$CI\% = \frac{\text{radioactivity of the test sample}}{\text{radioactivity of the control sample}} \times 100$$

Tumour inplantation test was performed by intracutaneous inoculation of $2 \times 10^5$ living hepatoma cells into the skin of spine. The average diameter of the tumours was measured at given intervals until days 25—28 post-inoculation (p.i.). The number of palpable tumours was counted and the death of the animals was registered. The volume of the tumours was calculated from $V = 0.4\, a \times b^2$, where a and b corresponded to the largest and smallest diameter of each tumour. The results were evaluated by Student's test.

## *Results and Discussion*

Within 3 hr p.i. of *C. burnetii* antigen to mice we found a considerable increase of virus-inhibiting activity in the serum which resisted heating to 56 °C, pH 2 and was considered for IFN-like. The virus-inhibiting activity in the serum culminated by 6 hr p.i. and then it decreased (Fig. 1) being unrecognisible at 72 hr p.i. in comparison with noninoculated mice. At this interval the IFN titre was lower than 3 $\log_2$.

Inoculation of the *C. burnetii* antigen to mice caused an increase of NK cell activity (Fig. 2). The cytotoxic index of their splenocytes increased by 6 hr p.i. to 42.9 + 9.2 as compared to 10 + 2.3 of noninoculated controls. Later on, between days 1—4 p.i., the cytotoxic index of splenocytes from the inoculated mice fluctuated but it did not differ essentially from that at day 6

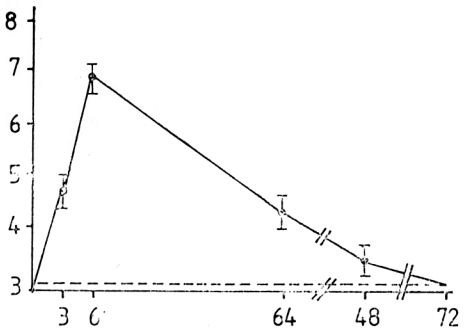


**Fig. 1**

IFN-like activity in the sera of mice which were give *C. burnetii* antigen
Abscissa: hr post-inoculation; ordinate: IFN-like activity ($\log_2$ of the dilution reciprocals).
Full line: inoculated mice; interruped line: control mice.

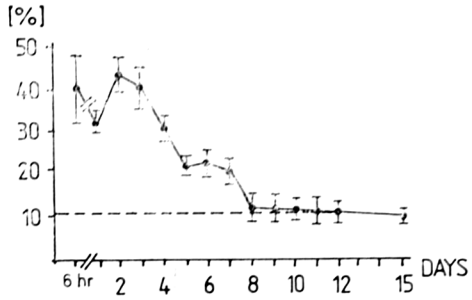


**Fig. 2**
Activity of NK cells in mice which were given *C. burnetii* antigen
Abscissa: days post-inoculation; ordinate: cytotoxic index (%).

p. i. Thereafter the activity of splenocytes decreased, but on day 7 p.i. it has still two times exceeded the control index. Since day 8 p.i. the cytotoxic index of splenocytes from antigen-inoculated mice did not differ from controls. The dynamics of IFN titres and of the CI of NK cells was similar to that described by others (Brezina *et al.*, 1968; Macela *et al.*, 1985).

Because the NK cells are thought to represent the first line of defense against tumour growth, we attempted to relate their activity to the proliferation of hepatoma 22a cells in mice. When hepatoma cells were inplanted by 43 hr after *C. burnetii* antigen inoculation, i.e. at the time when the NK cell activity has increased, the mice showed prolonged survival, delayed tumour growth and later occurence of the palpable tumours (Fig. 3). In con-

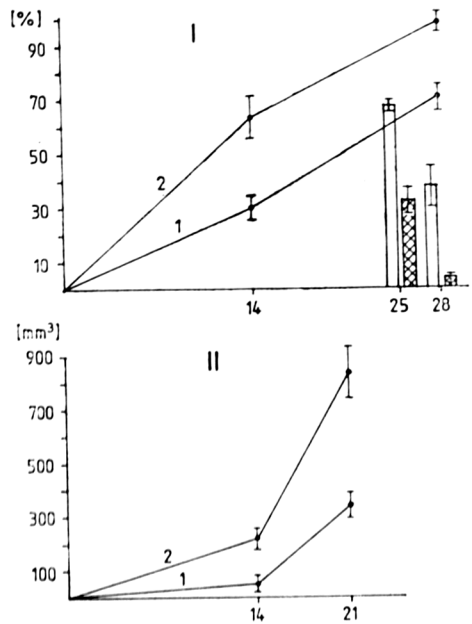


**Fig. 3**
Results of tumour cell inplantation in mice which were given *C. burnetii* (I) and in control mice (II)
I - Abscissa: days post-inplantation; ordinate: number of mice (%).
Curves show the percentage of mice developing tumours in the *C. burnetii* antigen recipients (1) and in the controls (2).
Empty columns: percentage of immunized survivors; hatched columns: surviving controls(%).
II - Abscissa: days post-inplantation. ordinate: volume of the tumours ($mm^3$); Each point represents the mean of 5 measurements in 10—12 mice.

trast, when leukosis cells were inplanted at intervals showing unchanged NK cell activity, i.e. since day 8 post-injection of the *C. burnetii* antigen, no influence on the growth of the tumour cells was noticed.

It seems, therefore, that inoculation of the *C. burnetii* antigen extract may influence the growth of hepatoma 22a cells in mice. The results allow to assume that the increased resistance to tumour cells in mice which had been given the *C. burnetii* antigen could be explained by at least two related circumstances: induction of IFN-like activity and an increased NK cell activity. After inoculation of the *C. burnetii* antigen, the mice developed elevated IFN levels in their serum culminating by 6 hr p.i. It is known that IFN as immunomodulator influences the activity of NK cells enhancing the nonspecific defense (Chetverikova and Polyak, 1987). We observed a quick increase of NK activity (already at 6 hr p.i. of the antigen) which may have been associated with the influence of IFN. The supposed role of NK cells is underlined by the fact that the MG 22a cells are especially sensitive to their cytotoxic effect (Malgin and Aprelikova, 1982). However, another mechanisms of antitumour defence could also be elicited by application of the *C. burnetii* antigen such as stimulation of mononuclear phagocytes (Kelly, 1977).

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