Time Course of the Content of Cellular Elements in the Peritoneal Exudate of BALB/c Mice Infected with Coxsackievirus A13

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Coxsackievirus A13 is shown to suppress macrophage exudation into the peritoneal cavity of adult male BALB/c mice. The influence of the infection on the counts of lymphocytes and polymorphonuclear leukocytes is less expressed. The detected changes are regarded as a manifestation of the immunomodulating action of coxsackievirus A13 infection.

Key Words: *coxsackievirus A13; BALB/c mice; peritoneal exudate; cell composition*

Coxsackieviruses play an important role in human pathology [7]. At present numerous reports point to a variety of pathogenetic mechanisms contributing to lesions induced by these viruses both during the natural course of infection in humans and during experimental infection in mice. Cell-mediated immunity factors are among the leading ones in the pathogenesis of coxsackievirus infection [1,5,6]. A nonspecific inflammatory exudate in the abdominal cavity may be easily induced by intraperitoneal injection of any stimulant. After 3 days the exudate consists mainly of mononuclear cells, with macrophages predominating. Intraperitoneal injection of a specific antigen at this time causes a rapid reduction in the number of macrophages in the exudate. Some scientists believe [4] that the reaction of the disappearance of peritoneal exudate cells is similar to the macrophage migration inhibition test *in vitro*. It may therefore be used for the assessment of cellular immunity.

This work was aimed at investigating the influence of coxsackievirus A13 on the time course of the content of cellular elements in the peritoneal exudate of BALB/c mice.

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MATERIALS AND METHODS

The studies were carried out on 150 male BALB/c mice aged 4 months from the *Stolbovaya* breeding center, Russian Academy of Medical Sciences. The prototype coxsackievirus strain A13 (Flores strain) was used, obtained from the Research Institute of Viral Preparations, Russian Academy of Medical Sciences (Moscow). The virus was replicated and titered in a monolayer culture of primary trypsin-treated human embryo fibroblasts by routine methods.

Four percent potato starch was used as a reagent stimulating the release of cellular elements into the abdominal cavity. The necessary amount of starch was brewed in distilled water, brought to the boil, autoclaved at 0.5 atm for 20 min, and frozen. Before use, the gel was thawed at room temperature. The resultant suspension was homogenized in a sterile glass homogenizer. One ml (40 mg) of this suspension was intraperitoneally injected to mice.

The animals were sacrificed by cervical dislocation. Peritoneal exudate cells were obtained by washing the peritoneal cavity with 8 ml of Hanks' solution with 5 units/ml heparin. The total content of nucleus-containing cells and their differentiated numbers were counted in the exudate. For this purpose, the cells were sedimented by centrifu-

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TABLE 1. Accumulation of Starch–Induced Inflammatory Cells in 3–Day Peritoneal Exudate of Male BALB/c Mice Infected with Coxsackievirus A13 ($M \pm m$)

Interval between infection and subsequent injection of starch, days	Total number of cells in peritoneal exudate, $\times 10^4$		Macrophage count, $\times 10^4$		Lymphocyte count, $\times 10^4$		Polymorphonuclear leukocyte count, $\times 10^4$	
	Coxsackie A13	medium 199	Coxsackie A13	medium 199	Coxsackie A13	medium 199	Coxsackie A13	medium 199
0	2058±122* (4)	2476±155** (4)	1589±95*	1951±117**	320±33	346±31	148±49	178±45
4	2876±362 (4)	2459±98 (4)	2368±411	2092±120	331±31	303±19	177±91	64±26
14	1812±126* (4)	2806±145** (4)	1306±106*	2316±85**	287±67	412±31	131±41	78±33
Intact mice after injection of starch	3056±292 (6)		2658±239		297±43		101±22	

Note. An asterisk shows differences reliable in comparison with analogous parameters for mice injected starch alone; two asterisks show differences reliable in comparison with analogous parameters for mice injected medium 199 and starch. In parentheses: number of animals per point of investigation.

gation (1000 rpm for 10 min), the supernatant was discarded, and the sediment resuspended in 3 drops of 50% cattle blood serum in Hanks' solution, after which smears were prepared which were stained with azure-eosin after Romanowsky. The percentage of macrophages, lymphocytes, and polymorphonuclear leukocytes was determined on the basis of scintillation of 200 cells [2]. The reliability of the results was assessed using Student's t test. Table 1 presents the arithmetic means and their errors for 4 animals.

RESULTS

Injection of 4% sterile starch in the peritoneal cavity of 4-month-old BALB/c mice caused the appearance of inflammatory exudate. Peritoneal flushes of intact mice contained $422 \pm 59 \times 10^4$ cells. Of these, 68% were macrophages ($289 \pm 53 \times 10^4$), 31% lymphocytes ($129 \pm 35 \times 10^4$), and less than 1% were polymorphonuclear leukocytes ($4.1 \pm 2.8 \times 10^4$).

Twenty-four hours after injection of the irritant, a reliable increase of the number of cellular elements was observed in the abdominal cavity, in comparison with intact animals ($p<0.05$). After 3 days the number of cells increased still more ($p<0.001$). Analysis of the time course of the content of specific types of cellular elements showed a marked increase in the number of polymorphonuclear leukocytes ($695 \pm 101 \times 10^4$, $p<0.01$) as early as 24 h after starch injection. After 72 h their number sharply decreased to $106 \pm 26 \times 10^4$, although it still remained higher than in the control ($p<0.01$). Changes in the levels of lymphocytes were more moderate. Nonetheless, after 72 h their content was reliably higher in comparison with intact animals ($p<0.01$).

Quite a different picture was observed with macrophages. Their content reliably surpassed the control level only on days 3-4 ($p<0.001$). During this period macrophages comprised the bulk (85%) of exudate cells, and for this reason the capacity of BALB/c mice to accumulate macrophages in the abdominal cavity was assessed 72 h after injection of the irritant.

For a study of the effect of coxsackievirus A13 infection on peritoneal exudation, 4-month-old BALB/c mice were inoculated with the virus in a dose of $10^{4,6}$ TCD_{50}/ml; this was associated with a depression of starch-induced migration of cells into the abdominal cavity (Table 1). A reliable inhibition was observed after starch injection on days 0 ($p<0.05$) and 14 ($p<0.01$) in comparison with the results observed after injection of the irritant alone. Similarly, a statistically reliable depression ($p<0.01$) was observed in infected mice after injection of starch on day 14 and in comparison with mice injected medium 199 instead of the virus. These changes were mainly due to macrophages. A reliable reduction in the quantity of these elements was observed on days 0 ($p<0.05$) and 14 ($p<0.01$) *vs.* analogous data for animals injected the irritant alone and on day 14 ($p<0.001$) for those injected medium 199 and the irritant.

The levels of lymphocytes and polymorphonuclear leukocytes remained at the baseline level over the entire period of observation.

Our results indicate that inoculation of BALB/c mice with coxsackievirus A13 affects the cellular inflammatory reaction in the abdominal cavity in response to a nonspecific irritant. A similar picture was observed when mice of the same strain were infected with coxsackievirus B3 [3]. In both cases we observed the minimal amount of macrophages in the exudate during the early periods after infection. The detected effect of inoculation may be due to changed chemotactic activity of the macrophages, which is one of the ways

in which coxsackievirus infection acts upon immunity [3].

Many viruses are known to induce a nonspecific immunomodulating effect by suppressing or boosting the immune response to a heterologous antigen [8]. Different groups of viruses have been studied to different extents in this respect. Our knowledge of the immunomodulating effects of enteroviruses, and specifically of coxsackieviruses, is particularly scanty [1,3]. The present findings demonstrating the ability of coxsackieviruses A13 and B3 to suppress macrophage exudation in the peritoneal cavity of infected adult mice confirm the immunomodulating effect of these viruses.

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