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TRANSFER OF DELAYED HYPERSENSITIVITY THROUGH REPEATEDLY SENSITIZED PERITONEAL EXUDATE CELLS DURING EXPERIMENTAL ANCYLOSTOMIASIS IN MICE

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Abstract. Current investigations using sensitized peritoneal exudate cells from mice infected repeatedly with *Ancylostoma caninum* larvae supply the evidence that these cells have the capability to transfer passive immunity to isogeneic recipients in contrast to normal cells from unifected donors. High doses of sensitization to donors and a lapse of time between the expansion and proliferation of cells in the recipients provide a rather strong imumnological readiness to expel/destroy the worm burden. The larvae bore through the gut reaching the peritoneal cavity but cannot enter the liver and/or lungs; they are thus misled (in the absence of normal guiding influences) to muscles where they undergo allergic death.

A passive transfer of adoptive immunity in recipients, as assessed by significant expulsion of worm burden utilizing sensitized peritoneal exudate cells (PEC) from infected donor mice, was achieved by Larsh et al. (1964) during *Trichinella spiralis*, Lang et al. (1967) during *Fasciola hepatica* and by Vardhani and Johri (1978, 1981a) during *Ancylostoma caninum* infections. In all these studies, larvae were either prevented from establishment (*T. spiralis*) or migration into liver (*F. hepatica*) and lungs (*A. caninum*), though in the latter case they readily migrated into the body musculature instead (Vardhani and Johri 1981a). A new vista was, however, opened up when it was through to investigate the effects of sensitized PEC (collected from repeatedly infected donors) in recipients challenged at 7 and 21 days after transfer in the *A. caninum* mouse model.

MATERIAL AND METHODS

Procurement of infective larvae, and collection and injection of sensitized and normal cells were done according to the methods reported previously (Vardhani and Johri 1981a). Three experimental donor groups A, B, and C of 6 to 8 weeks old isogeneic Swiss albino mice (weight 16—20 g) were infected orally with repeated doses of 125 + 125 + 250, 250 + 250 + 500 and 500 + 500 + 1000 larvae respectively at 7-days interval. A control group (D) of mice was kept as normal (unifected) for comparison. Repeatedly sensitized (from infected donors) and normal cells (PEC) were collected from A, B, and C 21 days after initial and 7 days after the last stimulating infection and from D simultaneously and injected (26×10^4) intraperitoneally into each of the six experimental recipients (a, a_1, b, b_1, c and c_1) and control (d) groups with 45 mice each. Each mouse of groups a, b, c and d was challenged at 7 days and that of a_1, b_1, and c_1 at 21 days after cell transfer and necropsied at 4 hourly intervals up to 24 hours and after every 24 hours up to 216 hours. Larval recoveries were made through Baermann's process after digestion in artificial gastric juice.

RESULTS

The total larval recovery was maximum in both experimental and control recipients at 4 hours after challenge in relation to the later recoveries (Fig. 1). Experimental groups showed lesser larval recoveries throughout the experimental period in

comparison to controls. Maximum expulsion or destruction of larvae occurred in groups b_1 (76.4 %) and c_1 (75.4 %) at 4 hours, expulsion of only 28.0 % in group a, and of 5.4 % in controls. The larval expulsion or destruction occurred rather rapidly in groups b, c, b_1 and c_1 till the whole larval burden reached zero level (at 48 hours in groups c and c_1, at 72 hours in group b_1 and at 96 hours in group b). Thus, the number of larvae (11.4 %) expelled was highest in group c from 16 (14.0 %) to 20 (2.6 %) hours and significantly, groups c and c_1 expelled or destroyed the whole larval burden

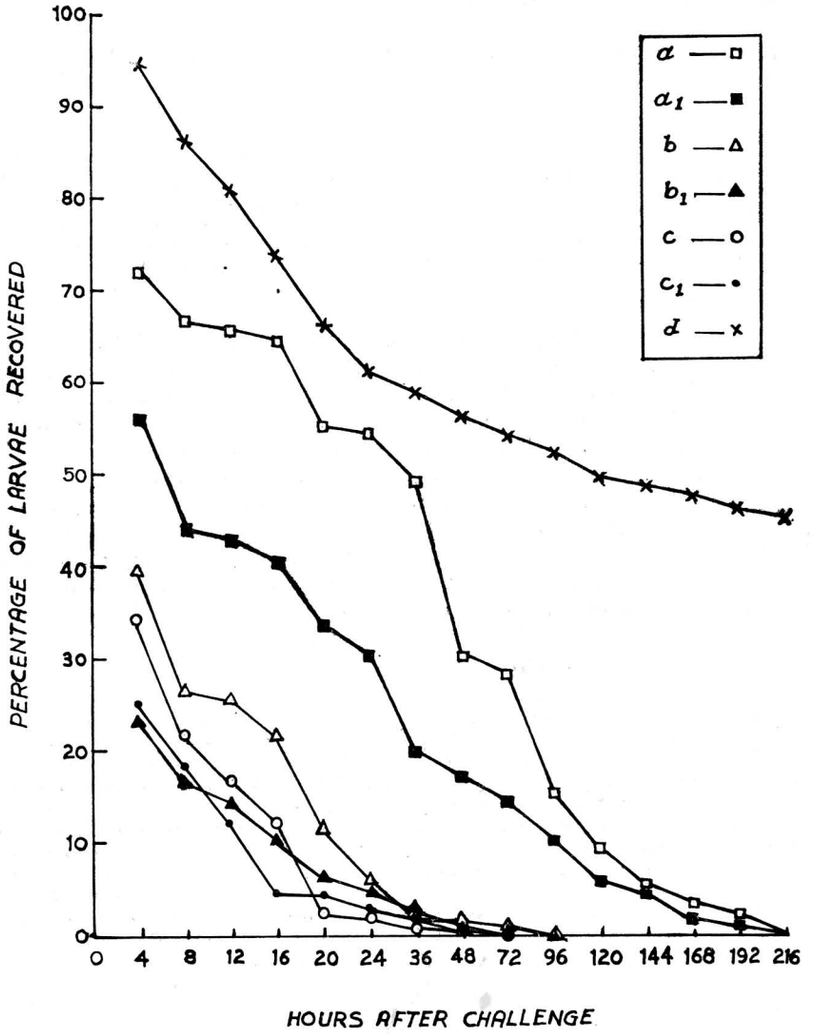

Fig. 1. Total percentage of *A. caninum* larvae recovered from experimental and control recipient mice after a challenge infection of 500 larvae at 7 (a, b, c and d) and 21 (a_1, b_1 and c_1) days after cell transfer. (Values are expressed in mean derived from 3 animals.)

earlier and more rapidly (by 48 hours after challenge) than all the other groups.

The gastrointestinal tract (GIT) retained maximum larval burden at 4 hours after challenge in groups a (67.2 %), b (36.2 %) and a_1 (44.8 %) and among all experimental groups, it became devoid of its entire larval burden by 20 hours in mice of group c_1, by 24 hours in groups b, c, b_1, by 96 hours in a_1, and by 120 hours in a. Interestingly, larval migration into lungs, heart, spleen, kidneys and brain has never occurred in the experimental animals during the entire experimental period. However, lungs and brain were invaded by the larvae at 24 (5.8 %) and 96 (0.2 %) hours after challenge in control mice.

It is interesting to note that larval migration into body muscles took place already within 4 hours after challenge in experimental groups c (4.4 %) and c_1 (3.4 %), within 8 hours in group a_1 (5.2 %), within 20 hours in group b_1 (1.2 %), within 24 hours in group b (6.0 %) and within 72 hours in group a (3.8 %) and at 24 hours in group d (0.6 %). The threshold larval burden in muscles was destroyed within 36 to 48 hours in b_1, 72 to 96 in c and 192 to 216 in a and a_1 groups. Threshold larval burden in controls (group d) was 45.8 % at 216 hours, i. e., at the end of the experimental period.

DISCUSSION

It has already been reported that singly sensitized peritoneal exudate cells produced significant immunity in recipients when compared to normal unsensitized cells (Vardhani and Johri 1981a). The present results indicate that a strong immune response can also be induced through repeatedly sensitized cells. Here, reinfection at 7 and 14 days to donors seems to have produced weak reaction. Dineen and Adams (1971) demonstrated that in repeatedly infected donors, the subsequent infections were expelled and the first one, being smaller, was unable to stimulate effectively the donor's immune system. Thus, cells collected from these donors on 21st day (7 days after the last stimulating infection) could not respond as effectively as the cells from singly sensitized donors and, therefore, produced a comparatively low immune response. Such cells also took longer time to stimulate the production and action of pharmacological mediators and/or lymphokines thereby delaying the whole process of expulsion and/or migration as well (Vardhani and Johri 1981b). The present results also indicate that groups b_1 and c_1 challenged 21 days after cell transfer produced maximum immune response eliminating 76.4 % and 77.4 % of larval burden within 4 hours in comparison to other groups challenged 7 days after cell transfer. This provided some sort of immunological readines to share the onus of eliminating at least a part of the larval burden.

The recoveries of larvae from GIT suggest that their total expulsion from recipients was found to be the same irrespective of the sensitization dose (light and heavy) to donors; within a period of 4 hours (from 16 to 20, the GIT got devoid of its larval burden in group c and from 20 to 24 hours in c_1) and 24 hours (from 72 to 96 in a and 96 to 120 in a_1) challenged at 7 and 21 days respectively after cell transfer. Such an expulsion would mean that all these experimental animals have acquired an immunological balance acquitting themselves to detect and expel larval burden during the initial hours of challenge, the time interval between cellular expansion, proliferation and release of antibodies providing the requisite influence along with increase of mast cells and histamine levels culminating in an overall expulsion process. Other factors include the increase of gut acidity and interactions between donated and recipient cells.

Interestingly, larvae that bored through the gut wall ultimately reached the peritoneal cavity (unpublished data) but could not proceed to liver; presumably due to the nature of the inflammatory fluid and its epithelial cells laden with antibody-like molecules acting as potential barriers. Thus the question of larvae migrating to lungs did not arise and it provides evidence for their bulk loss, also observed by Kerr (1938) and Lee (1960) during *Ascaris lumbricoides suum* infection in guinea pigs and *Toxocara canis* infection in mice. Finding no way to migrate to liver (and lungs), the larvae readily went into body musculature where they were ultimately trapped and destroyed because firstly they had no sojourn in the lungs which was essential; secondly they had been immunologically damaged in the intestine and thirdly they had probably lost the requisite oxygen tension for survival. The larvae were unable to migrate to certain other organs (spleen, kidneys and brain) also presumably because of unfavourable conditions in both experimental and control recipients; this was also reported by Bhopale and Johri (1975).

In case of *T. spiralis* infection in mice, Wakelin and Lloyd (1976) could not find rapid elimination of challenge infection through repeatedly sensitized mesenteric lymph node cells, similar to that observed in *A. caninum* mouse model. Some of the cells acting in the process may be carrying an antiparasite memory triggering off a series of actions imparting complexity to the system (Vardhani and Johri 1981a). It is evident from the rapid expulsion and/or destruction of larvae in experimental mice that the migratory behaviour and pathway of the challenged larvae have undergone a definite change.

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ПЕРЕДАЧА ЗАМЕДЛЕННОЙ ПОВЫШЕННОЙ ЧУВСТВИТЕЛЬНОСТИ ЧЕРЕЗ ПОВТОРНО СЕНСИБИЛИЗОВАННЫЕ КЛЕТКИ ПЕРИТОНЕАЛЬНОГО ЭКСУДАТА ПРИ ЭКСПЕРИМЕНТАЛЬНОМ АНКИЛОСТОМОЗЕ У МЫШЕЙ

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Резюме. При помощи сенсибилизованных клеток перитонеального эксудата от мышей, повторно зараженных личинками *Ancylostoma caninum*, было доказано, что эти клетки обладают способностью передавать пассивный иммунитет изогенным реципиентам, в отличие от нормальных клеток от незараженных доноров. Высокие дозы сенсибилизации доноров и промежуток времени между экспансией и пролиферацией клеток в реципиентах вызывают довольно сильную иммунологическую готовность выделять или разрушать личинки. Личинки проникают через кишку в брюшную полость, но не могут пробраться в печень или легкие. Таким образом они ошибочно направлены (в отсутствии нормальных направляющих влияний) в мышцы, где погибают от аллергии.

REFERENCES

BHOPALE M. K., JOHRI G. N., Experimental infection of *Ancylostoma caninum* in mice. II. Migration and distribution of larvae in the tissues after oral infection. J. Helminthol. 49: 179—185, 1975.

DINEEN J. K., ADAMS D. B., The role of recirculating thymus dependent lymphocytes in resistance to *Trichostrongylus colubriformis* in the guinea pig. Immunol. 20: 109—113, 1971.

KERR K. B., The cellular response in acquired resistance in guinea pigs to an infection with pig ascaris. Am. J. Hyg. 27: 28—51, 1938.

LANG B. Z., LARSH J. E., WEATHERLY N. F., GOULSON H. T., Demonstration of immunity to *Fasciola hepatica* in recipient mice given peritoneal exudate cells. J. Parasitol. 53: 208—209, 1967.

LARSH J. E., GOULSON H. T., WEATHERLY N. F., Studies on delayed (cellular) hypersensitivity in mice infected with *Trichinella spiralis*. II. Transfer of peritoneal exudate cells. J. Parasitol. 50: 496—498, 1964.

LEE H. T., Effects of superinfection on the behaviour of *Toxocara canis* larvae in mice. J. Parasitol. 46: 583—588, 1960.

VARDHANI V. V., JOHRI G. N., Rejection of worm load through singly and repeatedly sensitized peritoneal exudate cells during experimental ancylostomiasis. Experientia 34: 123—124, 1978.

—, —, Migratory behaviour and survival pattern of *Ancylostoma caninum* larvae in an adoptively immunized host. Int. J. Parasit. 11: 145—147, 1981a.

—, —, Pharmacological estimation of histamine in the intestine of mice experimentally infected with repeated doses of *Ancylostoma caninum* larvae. J. Hyg. Epidemiol. Microbiol. Immunol. 25: 31—34, 1981b.

WAKELIN D., LLOYD M., Accelerated expulsion of adult *Trichinella spiralis* in mice given lymphoid cells and serum from infected donors. Parasitology 72: 307—315, 1976.

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