30×10^4 thymus cells from donor groups A and B were injected i.p. into each mouse of the recipient groups a_1 and b_1 (each having 24 mice of same age and weight) respectively. Similarly recipient groups a_2 and b_2 also with 24 mice each received 50×10^4 bone marrow cells from the same donor groups. Control recipient groups c_1 and c_2 received thymus (30×10^4) and bone marrow (50×10^4) cells respectively from uninfected donor group C mice.

Each recipient mouse of all 6 groups was orally challenged with a single dose of 500 *A. caninum* larvae 7 days after cell transfer. Necropsies were made from different organs and muscles of mice initially at 6-h intervals following Baermann's technique after 3 h digestion in artificial gastric juice. The number of actively motile larvae recovered at each necropsy was recorded and results with their statistical analysis are presented in the table.

Result and discussion. Significant expulsion of the larvae took place in recipient groups a_1 (48.0%) and a_2 (47.3%) which received cells from donors sensitized with 1000 larvae in comparison to groups b_1 (31.4%) and b_2 (39.5%) which received cells from donors sensitized with 500 larvae at 6 h after challenge. Thereafter the percentage of larvae expelled increased rapidly with time. In groups a_1 and a_2 rate of decline in the number of larvae recovered was rather rapid, resulting in the total expulsion and/or destruction of larvae by 144 h (6th day) and 120 h (5th day) respectively. Recipients injected with cells from donors (group B) were more competent in expelling larvae from a challenge dose than the recipients which received unsensitized cells from control donors (group C). These results are statistically significant.

The results are surprising as they indicate clearly that sensitized thymus and bone marrow cells are able to confer immunity passively in *A. caninum* mouse model. These cells seem to play their role in CMI similar to that of mesenteric lymph node and peritoneal exudate cells in this model[17,18]. The expulsion of larvae from the gastrointestinal tract (which took place at 48 h after challenge) was found to be due to severe inflammation and to infiltration of sensitized cells. The release of histamine[21] furthermore adds to the conditions unfavourable for the maintenance of the larvae. Lung migration in the present work was very marked [17,18]. This may account for the slow destruction of larvae in muscles[22] in this series of experiments, in contrast to the results of Vardhani and Johri[18] who observed the presence of larvae in muscles at 4 h after challenge. The larvae did not appear in muscles in this case until upto 24 h after challenge and, thereafter, they persisted in the muscles for a considerably long time (upto zero recovery) although they were not so active.

1 Acknowledgment. We are indebted to Professor B.M. Sinha for providing facilities and to Council of Scientific and industrial Research, New Delhi for funds.
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Immunological observations following vasectomy

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Summary. Lymphocytotoxic antibodies (LCA) against panels of normal lymphocytes and leukemic B-cells were demonstrated in vasectomized men. Since vasectomy is known to induce antibody formation to spermatozoa, the demonstration of these lymphocytotoxic antibodies may be related to antigenic constituents of spermatozoa such as HLA or B-cell alloantigens. Long term follow-up is needed to clarify the clinical significance of these antibodies.

In a previous study, 9 of 12 vasectomized men developed lymphocytotoxic antibodies (LCA) which were considered to be specific for, or at least cross-reactive with HLA antigens[2]. The present report of 10 additional subjects is based on a more extensive evaluation of LCA as tested not only against normal lymphocytes but also against leukemic B-cells. The occurrence of autoantibodies was also investigated.

Materials and methods. The study group comprised 10 men, aged 30–48 years, who had bilateral vasectomy under general anesthesia. Blood samples were drawn prior to vasectomy and at selected intervals following vasectomy for a period of up to 24 months. Blood samples were similarly drawn from age-matched controls comprising male patients who underwent minor surgical procedures and healthy male employees of the hospital. None of the 10 study cases nor any of the controls received blood transfusions prior to or during the course of study.

Lymphocytotoxic antibodies: Sera of the vasectomized men and of the control subjects were tested for LCA using a

Table 1. Lymphocytotoxic antibodies in vasectomized and control cases*

Group	Positive reactions/cases tested					
	Cases	Before surgery	After operation (months)			
			1–3	4–6	7–9	10–24
Vasectomy a	5	0	3.5	3.0	2.0	0.25
Vasectomy b	5	0.25–0.5	1.25	1.6	0	0.25
Surgical controls	7	0	0	0		
Non-surgical controls	10	0	0	0		

*LCA against panel of normal lymphocytes from 40 normal donors.

Table 2. Lymphocytotoxicity against leukemic b-cells before and after vasectomy*

Case	1	2	3	4	5	6	7	8	9	10
Months	0** 2 7 15	0 2 5 6 9	0 1 3 4 7	0 13	0 5 29	0 6	0 2 14	0 2 4 8 12 13	0 8	0 1 3 7
Leukemic lymphocytes										
CLL	. . 6-8.	. . . 8.	 8	. 8 8	8 8.	. 6	. 6-8 8	. . . . .	. .	. . . .
CLL	. . . .	. . . 6.	. . . . .	. . .	. . .	. 8	. . .	. . . . .	. .	. 4-6. .
CLL	. . . .	. . . . .	. . . . .	. . .	. . .	. .	. . .	. . . . .	. .	. . . .
CLL	. . . .	. . . . .	. . . . .	. . .	. . .	. .	. . .	. . . . .	. .	. . . .
ALL	. . . 6	. . . . .	. . . . .	. . .	. . .	. .	. . 6	. . . . 6 .	. .	. . . .

* Lymphocytotoxicity scored according to the percentage of killed lymphocytes as follows: 2 (10–19%), 4 (20–39%), 6 (40–79%), 8 (80–100%). Readings of ⩽ 39% killed lymphocytes are represented by dots. ** Denotes pre-vasectomy specimen.

panel of freshly prepared lymphocytes (comprising both T and B cells) from 40 healthy donors by the microdroplet method of Tarasaki and McClelland[3]. The sera of the vasectomized men were also tested for LCA (I) by a modified Amos technique[4] using a) B lymphocytes from 4 cases of chronic lymphocytic leukemia, b) cultured B cell lines and c) B cells from 11 normal donors; (II) by the NIH lymphocyte microcytotoxicity test[5] using B-cells from a case of acute lymphoblastic leukemia.

Autoantibodies: Antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) were tested by indirect immunofluorescence using standard techniques[6]. Appropriate controls were included for each test.

Results. Lymphocytotoxic antibodies: The sera of 6 of the 10 vasectomized men showed a significant increase of LCA activity against our 40-donor-panel of normal lymphocytes. This increase was noted at between 2 and 6 months after vasectomy; it decreased or even disappeared during the remainder of the 2 years studied (table 1).

As tested against leukemic B-cells, LCA activity was demonstrated from 1 to 13 months after vasectomy in all but 1 of the 10 cases (table 2). The reaction against the cultured B-cell lines was weak in 2 of the 10 study subjects. There was no reaction in any of the vasectomized men against B-cells from 11 normal donors. The sera of the vasectomized men tested for autoantibodies against their own lymphocytes gave weak reaction in 3 cases and no reaction in the others.

Autoantibodies: On ASMA assay a marked increase in titer, from 0 prior to vasectomy to 1:160 at 19 months afterward, was observed in 1 case and a mild increase in titer was noted in 2 others. A rise in ANA titer from 1:20 preoperatively to 1:160 at 24 months after vasectomy was seen in 1 case and a mild increase was noted in another. The control cases did not develop any significant titers of ASMA or ANA.

Discussion. The effect of vasectomy on the development of LCA activity against normal panel lymphocytes is summarized in table 1. Briefly, whereas the sera of pre-operative and post-operative controls were negative for LCA activity, only those of the men who underwent vasectomy exhibited any appreciable levels of LCA. Thus, the surgical procedure as such could not be incriminated for the induction of LCA activity. The development of LCA against normal lymphocytes in post-vasectomized subjects has been observed previously by us[2] and by others[7,8] and appears to be a transient response to vasectomy. As shown in table 2, almost all study cases (9/10) developed LCA activity against leukemic B-cells after vasectomy. The LCA activity was observed only against leukemic B-cells of 3 of 5 donors suggesting that LCA activity against B-cells appears to be restricted to, or possibly be specific for, B-cell alloantigens. Since it has been shown that vasectomy induces sperm agglutinating antibodies in 50–60% of vasectomized men[9-11] and since spermatozoa are known to be endowed not only with B-cell alloantigens[12] but also with HLA antigens[13], the LCA activity demonstrated in our study cases may be related to retention of spermatozoa and resorption of the antigenic constituents of spermatozoa[14-17].

It is noteworthy that in spite of strong reactions against leukemic B-cells the reaction against cultured B-cell lines was weak and present in only 3 cases; no reaction was demonstrated against normal B-cells. Such diverse reactions against different kinds of B-cells have been reported by others who have studied sera containing B-cell antibodies[18-20]. Other autoimmune antibodies[21] in addition to the LCA is evident from the titers of antinuclear and anti-smooth muscle antibodies of the sera of vasectomized subjects of this study. It remains for further study to elucidate the nature and clinical implication of induced autoimmune antibodies as a consequence of vasectomy.

1 We are indebted to Dr M. Fotino for performing some of the lymphocytotoxicity tests recorded in the text.
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