function. However, in addition to the B-cell function, the possibility of the virus affecting helper T-cell function cannot be ruled out as, in our earlier study, we have found that the virus suppresses the response to helper T-cell dependent antigen[8]. The present investigation also suggests that the immunosuppression is caused by the active multiplication of the virus and not by the viral antigen alone, since the administration of UV-inactivated virus failed to cause immunosuppression. This finding rules out the possibility in Dengue virus infection of the immunosuppression as noticed in certain viral infections to result from the competition of viral antigen with another antigen in the production of antibody, thereby suppressing the response to the latter antigen[13].

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Alteration of early T lymphocyte count in patients with herpes genitalis[1]

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Summary. Peripheral blood samples from 52 women, including 16 with herpes genitalis and 36 healthy persons, were studied to enumerate subpopulations of lymphocytes. It was found that the mean percentage of 'active' T lymphocytes was significantly less in the patients with herpes genitalis than in the controls.

Progenital herpes or cervical herpes is one of the most common infectious diseases in female genitalia and is caused by herpes simplex virus type 1 or type 2 (HSV-1 or HSV-2). Current studies suggest that cell-mediated immunity may play an important role in recovery from herpetic infection. The present study was designed to assess whether cellular immunity is depressed in patients with herpes genitalis, utilizing determination of T and B lymphocyte subpopulations in their peripheral blood.

Materials and methods. A total of 52 Japanese women, 16 with herpes genitalis and 36 healthy persons, were studied. Herpetic involvement was confirmed by viral isolation, exfoliative cytological examination or both. All patients received serological examination for HSV antibodies by the methods described previously[2]. 4 of the 16 sera had no antibodies against either HSV-1 or HSV-2 and were designated as primary infections. The remaining 12 sera had demonstrable antibodies against either virus and were considered to be secondary infections, although 11 of these patients had no previous history of herpes genitalis.

Lymphocytes were isolated from freshly drawn heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation[3], and the cells were adjusted to a final concentration of 2×10^6/ml in PBS containing 0.1% bovine serum albumine (PBS-BSA).

'Active' T lymphocyte rosette assay was done by the method described by Wybran and Fudenberg[4]. 'Total' T lymphocytes were determined by the method described by Jondal et al.[5].

'Total' B lymphocytes were assayed by immunobead rosette tests described previously[6,7] using polyacrylamide beads coated with anti-human immunoglobulin light chains ($\varkappa$ and λ). Immunobeads were obtained from Bio Rad · Laboratories (Richmond, California). Each bead population was suspended in PBS to give a concentration of 1×10^8 beads/ml.

Results. Normal percentages of total T lymphocytes were encountered in the patient group, whereas mean percentages of active T lymphocytes were less than those of the healthy controls, and the difference of active T lymphocyte counts between the 2 groups was statistically significant ($p < 0.001$) as shown in table 1. 7 of 16 (43.8%) with herpes genitalis had lower percentages of active T lymphocytes than the lowest limit of normal controls (normal range: 12.0–41.5%).

Mean percentages of total B lymphocytes are shown in table 2. The patient group showed no differences from controls in the percentages of total B lymphocytes.

Table 1. 'Active' and 'total' T lymphocytes in patients with herpes genitalis and in healthy women

	T lymphocytes (%)			
	Active	p	total	p
Healthy women	25.0±8.3		62.8±7.6	
Patients	13.9±6.8	<0.001	62.2±7.9	n.s.

Table 2. Total B lymphocytes in patients with herpes genitalis and in healthy women

	Total B lymphocyte (%)	p
Healthy women	14.0±4.2	
Patients	12.8±3.9	n.s.

Discussion. Sawanobori and co-workers[8] investigated the subpopulations of peripheral blood T and B lymphocytes in 11 patients with recurrent herpes genitalis utilizing SRBC rosette tests and described that patients with herpes genitalis had normal percentage of early rosettes (active T lymphocytes), total rosettes (total T lymphocytes) and EAC rosettes (total B lymphocytes). Our findings presented herein indicate, in contrast to those by Sawanobori et al., that percentages of active T lymphocytes are apparently lower than those of normal controls. This differene is, at least in part, due to the analytical methods employed; the previous authors used a SRBC: lymphocytes ratio of 8:1, while we used that of 27:1. Furthermore, the patients they studied were subject to recurrent herpes genitalis, whereas 15 of 16 patients in our series had no episode of herpes genitalis or labialis despite the presence of serum neutralizing antibodies[9,10]. Approximately 95% of Japanese females aged 31 or over carry serum neutralizing antibodies against either HSV-1 or HSV-2 without apparent episode of herpes labialis or genitalis. Accordingly, clinical or immunological features of herpes genitalis in Japanese women seem to be different from those in Caucasian or Negro women. The viral types involved in herpes genitalis also differ between Japanese women and Caucasion or Negro women. More than two thirds of the occurences of herpes genitalis in the USA are due to HSV-2, while in Japan approximately half are due to HSV-1[11,12]. These methodological or epidemiological differences may be responsible for the different results in the 2 studies, and our present findings agree with the description by Wybran and Fudenberg that all patients with viral disease had less than 15% rosette forming cells in the active T cell assay[13].

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Dietary immunostimulation: Interaction with BCG and LPS

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Summary. The action of BCG and LPS mR595 used in conjunction with a formula-defined diet is dependent on the administration timing and resembles that of interacting adjuvants affecting different elements of the immune system.

Production of anti-sheep erythrocyte (SRBC) antibody has been shown to be enhanced in rats fed an easily absorbed formula-defined diet (FDD), instead of the usual laboratory chow[2]. Such formulations may be used, in the clinic, to lessen the nutritional imbalance resulting from cancer and cancer therapy[3]. On the other hand, over the past years, BCG has been used as an adjunct against cancer in humans[4-6], and anti-tumour properties of bacterial lipopolysaccharides (LPS) have been investigated[7,8]. However, the fact that FDD may influence the immune response suggests that the action of adjuvants administered to FDD-fed organisms could be analogous to the interaction of different immunostimulants. Present studies were initiated to verify this hypothesis.

Virgin female Sprague-Dawley rats, 6–7 weeks old, were fed FDD 3[2] or Purina laboratory chow 5001 for 7 days, and challenged with SRBC alone or in conjunction with BCG (bovine strain, live, 1 mg i.p.) or LPS mR595 (*Salmonella minnesota,* 40 µg i.p.). Both concentrations were used in view of their known oncotherapeutic action[4,8]. Since the administration timing is known significantly to affect their action[4,7], the adjuvants were given either together with (concomittantly) or within minutes after (sequentially) SRBC. Logarithmic transformation of the titers was performed and mean titer values were obtained. Differences between the means were appraised using Student's t-test (N-2 degrees of freedom, 2-sided level of significance).

As previously reported, the FDD alone was responsible for more than a 7-fold increase of the anti-SRBC antibody titers (table). Administered sequentially, BCG provided no further immunostimulation than that provided by the diet ($p > 0.1$) while a 25-times increase was observed in the chow-fed controls ($p < 0.001$). As to sequential LPS mR595, the 4–5-fold enhancement ($p < 0.05$ for each dietary group) achieved was diet-independent, but did not hinder the stimulating action of the FDD. In fact, the LPS and the FDD had a compounded action. Administered concomittantly, the LPS was totally ineffective in both dietary groups ($p > 0.1$), while BCG provided a significant ($p < 0.01$ for both groups) and food-dependent antibody increase (6- and 3-fold, respectively in chow- and FDD-fed animals).

The adjuvant action of BCG and LPS mR595 is influenced by both the administration timing and the types of food eaten. Known to stimulate the reticuloendothelial system and the T-cells[9], BCG can also act similarly to LPS[9,10] which substitute for T-cells and stimulate B-lymphocytes[11,12]. This enables BCG to affect both cellular and humoral immunities while lipopolysaccharides influence primarily the humoral response. As to FDD3, it increases