IMMUNOMODULATING ACTIVITY OF SUNFLOWER OIL AND STARCH ADMINISTERED PERORALLY TO MICE

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Polysaccharides and some components of vegetable oils are known to have an immunomodulating effect not only on parenteral [5, 6], but also on peroral administration [8, 11]. For instance, peroral administration of β-glucan of microbial origin [11] or of polyunsaturated fatty acids [8] leads to intensification of phagocytic enzyme activity of macrophages. Among polysaccharides and vegetable oils, starch and sunflower oil are the principal fillers and solvents of water- and fat-soluble therapeutic preparations. Information on the immunomodulating properties of these substances could not be found in the accessible literature. Since all preparations made on a starch base and the majority of fat-soluble therapeutic substances are administered perorally, it is important to study the immunomodulating properties of starch and sunflower oil when administered perorally.

In this investigation, in order to study the mechanisms of action of starch and sunflower oil, their effects on the parameters of immunity were compared in intact and thymectomized animals.

EXPERIMENTAL METHOD

Experiments were carried out on 80 intact, 64 thymectomized, and 68 mock-thymectomized male CBA mice. Thymectomy was performed on mice weighing 12-14 g by a surgical method under superficial ether anesthesia [2]. All stages of the operation except removal of the thymus were carried out on animals undergoing the mock operation. The mice were used in the experiments 1 month after the operation. The age and body weight of the intact animals corresponded to the analogous parameters of animals undergoing operation. The following substances were tested: unrefined sunflower oil isolated from the residue by decanting, and 1% starch gel obtained by bringing the suspension up to boiling point. The reference preparation used was glutamic acid, which has marked immunostimulating activity [4]. The preparations were administered to the mice through a tube in a volume of 0.2 ml in the course of 10 days. Control (thymectomized, mock-thymectomized, and intact) animals were given physiological saline in accordance with the same schedule. The animals were then divided into a two groups: in one group (without immunization) the number of Thy-I^+-splenocytes was determined, the other group was immunized intravenously with sheep's red blood cells ($5 \cdot 10^6$; SRBC), and on the 4th day after immunization the following parameters were determined: the number of IgM-antibody-forming cells (IgM-AFC) in the spleen by the method of Jerne and Nordin [10], and the hemagglutinin titer in the serum. The number of IgM-AFC was expressed per 10^6 karyocytes. Thy-I^+-splenocytes were detected with the aid of rabbit antiserum against the cerebral cortex of CBA mice in the complement-dependent cytotoxic test [1]. Antiserum (1:50), absorbed with mouse liver, and with mouse and sheep red blood cells [1], in the presence of fresh guinea pig complement (1:3), led to death of 88.0 $\pm$ 1.3% of the thymocytes and did not interact with bone marrow cells of CBA mice. No fewer than 200 cells whose viability was estimated with the aid of a 0.2% aqueous solution of trypan blue were counted in each test.

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TABLE 1. Effect of Peroral Administration of Sunflower Oil, Starch, or Glutamic Acid on Number of Thy-I$^+$-Splenocytes of Thymectomized and Mock-Thymectomized Mice (M ± m)

Preparation	Number of Thy-I$^+$-splenocytes (cytotoxicity index of anti-bone-marrow serum, %)	
	thymectomized mice	mock-thymectomized mice
Physiological saline	2.5±1.0	27.2±2.4
Sunflower oil	47.7±3.5*	35.7±3.4*
1% Starch gel	29.0±3.2	23.0±2.9
Glutamic acid in physiological saline	27.6±2.1	22.5±2.9

Legend. Asterisk indicates significant difference compared with cytoxicity index of mice undergoing mock operation and receiving physiological saline ($p < 0.01$).

TABLE 2. Effect of Peroral Administration of Sunflower Oil, Starch, or Glutamic Acid on Immune Response to SRBC (M ± m)

Preparation	Mice					
	Intact		thymectomized		mock-thymectomized	
	number of IgM-AFC per 10^6 splenic karyocytes	reciprocal titers of hemagglutinins	number of IgM-AFC per 10^6 splenic karyocytes	reciprocal titers of hemagglutinins	number of IgM-AFC per 10^6 splenic karyocytes	reciprocal titers of hemagglutinins
Physiological saline	33.5±2.6 (10)	160.0±51.0 (10)	10.6±1.2 (8)	85.0±15.9 (8)	25.2±1.2 (8)	200±31.8 (8)
Sunflower oil	20.5±2.6 (10)*	155.3±66.0 (10)	8.7±0.8 (8)	80.0±15.9 (8)	17.0±0.9 (8)*	95.0±15.9 (8)*
1% Starch gel	41.3±6.5 (10)	272.0±51.3 (10)	7.7±0.9 (8)	70.0±5.3 (8)	29.5±0.8 (8)	220.0±21.2 (8)
Glutamic acid in physiological saline	50.2±5.3 (10)*	288.0±17.3 (10)*	28.0±3.9 (8)*	220.0±21.2 (8)*	46.5±5.5 (10)*	256.0±17.3 (10)

Legend. Asterisk indicates significant difference compared with corresponding control ($p < 0.01$). Number of animals given in parentheses.

EXPERIMENTAL RESULTS

As will be clear from Tables 1 and 2 peroral injection of glutamic acid into the thymectomized mice restored the normal number and functional activity of short-living splenic T-cells. Starch restored the number of Thy-I$^+$-splenocytes but, unlike glutamic acid, it did not alter the level of the immune response. The oil significantly ($p < 0.01$) increased the number of Thy-I$^+$-cells compared with the value for the animals undergoing the mock operation: like starch, it did not change the immune response of the thymectomized mice.

Feeding the mock-thymectomized or intact mice with glutamic acid potentiated the immune response. Starch did not change the immune response of these groups of animals, whereas the oil suppressed it (Table 2).

The results show that, like the highly immunoactive glutamic acid, starch and oil, when administered perorally to thymectomized mice, can induce the Thy-I-antigen on precursor T cells. Induction of Thy-I-antigen under the influence of chemically different substances confirms and adds to information in the literature [3] on the nonspecific character of this phenomenon. Induction of Thy-I-antigen by starch with no effect on the immune response is in agreement with data in the literature [9] on the possible absence of correlation between the appearance of the Thy-I-marker on the cell and the change in its function. Suppression of the immune response under the influence of the oil accompanied by simultaneous induction of Thy-I-antigen on precursor T cells is evidence that correlation between the appearance of the Thy-I-antigen on the cell and immunostimulation is not always present. The inhibitory

effect of the oil may be determined by fatty acid metabolites: prostaglandins which, as we know [6, 8], have an immunosuppressive action.

The data given above are important when the results of research into the immunostimulating activity of fat-soluble vitamins A and E, in which the immune activity of the oil was not monitored, are analyzed [5, 7]. The results of the present investigation are important both for an understanding of cardinal questions in the immunology of nutrition, and for the search for new approaches to the regulation of immune homeostasis

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