OCCURRENCE OF PLAQUE-FORMING CELLS AND ANTIBODIES IN THE CERVICAL MUCUS, BLOOD AND MILK DURING THE OESTROUS CYCLE OF COWS IMMUNIZED WITH SHEEP ERYTHROCYTES

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Cows, being in the luteal or follicular phase of the oestrous cycle, received intrauterine (4 cows) or intravenous (2 cows) injections of sheep red blood cells. Plaque-forming cells (PFC) occurred in the cervical mucus, blood and milk regardless of the route of the antigen administration and phase of the oestrous cycle. However, cow immunized intravenously in the follicular phase displayed higher number of PFC in blood than cow immunized in the luteal

phase. In cows which were given intrauterine inoculation of the antigen in the follicular phase, PFC occurred in the cervical mucus up to the 25th day of observation. In cows injected in the luteal phase, the number of PFC showed 2 "peaks" coincident with follicular phase of the cycle.

The uterine cervix is biologically similar to such glands as the salivary glands, lacrimal glands or mammary gland, which possess the ability to secrete IgA antibodies and which were called "the first defense line of the organism". Also, the cervical mucus usually contains lactoferrin which is associated with an autonomic capacity of tissues for the defense (1).

It was found that defense reactions of the genital tract depend on the cycle phase (3, 4, 6). In oestrus an increased permeability of the capillary endothelium leads to a heavy leucocytic infiltration of the uterine wall, accumulation of leucocytes in the uterine lumen and intensive phagocytic and bactericidal activity. In this phase, a rapid passage of bacteria through the endometrium to the regional lymph nodes mobilizes the leucocytic system to defense (5, 7). In the luteal phase, however, microorganisms do not pass through the endometrium, multiply in its lumen and produce endometritis. It is considered that endometrial impenetrability for bacteria delays leucocytic response to infection. Leucocytes occur later and their phagocytic activity is not so efficient as in the former phase (2).

It was found that oestrogens suppress immune response in mice immunized with sheep red blood cells, particularly when the reaction was measured by the number of plaque-forming cells in the spleen rather than by serum antibody level (8). There are suggestions that the depression of the immune response to the antigen administered results from the stimulation of the RES by oestrogens and the diversion of the antigen from sites where the immune response is initiated.

The present study intended to perform observations on plaque-forming cells (PFC) in the cervical mucus, blood and milk of cows after intravenous or intrauterine injection of sheep red blood cells (SRBC) in various phases of the oestrous cycle.

Material and Methods

The studies were performed on 6 Friesian-Holstein multiparous cows orginating from a herd of 60 dairy cows, free from brucellosis and tuberculosis. The selection of cows being in the same oestrous cycle phase was easy because a number of animals often exhibited oestrus at the same day. Three cows were immunized in the follicular phase of the oestrous cycle: two by intrauterine route (i.u.) and one intravenously (i.v.). The same number of animals was immunized in the luteal phase: two cows i.u. (4 days after oestrous) and one i.v. (12 days after oestrus). Cows immunized i.u. were each given 200 ml of 25 per cent SRBC and cows injected i.v. — 20 ml of 20 per cent SRBC. Blood, milk and cervical mucus were collected for the examinations.

One ml of blood was taken from the jugular vein, mixed with 0.6 ml of heparin and cooled. After centrifugation, the buffy coat was removed and washed twice with Parker's solution containing 10 per cent calf serum. After the last centrifugation, the leucocytes were resuspended in 1 ml of Parker's solution and kept in ice till the use.

Mucus samples were taken, using a glass tube, from the cervical orifice. Then the mucus was transferred to a tube with Parker's solution (1 ml) and kept in ice-

water till the use. When its consistency was too viscid, the mucus was ground in a glass mortar before plating.

Plaque-forming cells (PFC) were assayed by use of the Jerne technique modified by Sterzl (11). Three-tenth ml of the examined material was mixed with 0.6 ml of melted agar and dropped on Petri's plates. After incubation at 37°C for 18 hours, guinea pig serum (complement) diluted 1:10 was added and the plates were incubated at 37°C for an hour. Plaques whose number corresponded to that of antibody-forming cells, were counted with a dissecting microscope. The number of PFC was calculated per million lymphoid cells.

Serological examinations were performed on the material inactivated at 56°C. The haemagglutination test was made using sheep red blood cells as antigen (1 drop of 1 per cent SRBC was added to 0.5 ml of the diluted appropriately material). The samples were incubated at room temperature for 2 hours and then overnight at 4°C.

After recording the results of the haemagglutination test, 0.1 ml of complement, diluted 1:10, was added to each tube. The samples were kept in a 37°C water-bath for half an hour and then the results were read.

Results

Cows immunized i.v. in the follicular phase of the cycle developed blood PFC 2 days after administration of the antigen, whilst cows injected in the luteal phase — one day later. When the antigen was inoculated i.u., PFC also appeared in blood regardless of the time of immunization. The number of PFC was distinctly higher after i.v. immunization than that after i.u. inoculation. On the 3rd day of observation the number of PFC was over 2.5 times higher in the cow inoculated i.v. in the follicular phase than in the cow immunized in the luteal phase. Moreover, in the cow injected in the cow injected in the follicular phase, PFC were observed up to the 9th day after immunization (Table 1).

The occurrence of PFC in the cervical mucus is presented in Table 1. As can be seen, in cows immunized i.v. PFC in mucus were observed throughout the same period as that in blood, i.e. up to the 9th day after immunization, whereas in cows inoculated i.u. — for 25 days. In cows immunized in the luteal phase, the number of PFC showed two peaks: the first at 3—5 days after immunization and the second between 10 and 13 days i.e. before the following oestrus.

It was found that the PFC response in the cervical mucus was seen in every cow regardless of inoculation route of the antigen. In cows injected iv. in the follicular phase, PFC appeared a day earlier than after intrauterine immunization. The number of PFC detected in the cervical mucus of these cows was independent of administration route of the antigen.

Cows immunized in the luteal phase developed PFC in the cervical mucus at 3 days after inoculation and the number of PFC was noticeably lower after intravenous immunization than that after the intrauterine (Table 1).

The PFC in milk occurred also in all the cows examined. They were usually detected at the same time as in blood, i.e. between 3 and 7 days after immunization, but their number was lower than that in blood. Cows injected iv. in the follicular phase developed more PFC than animals immunized in the luteal phase. On the other hand, after intrauteri-

Table 1

Appearance of PFC in blood, cervical mucus, and milk of cows immunized with sheep red blood cells in various phases of the oestrous cycle

No. of cow	Phase of oestrous cycle	Route of antigen administration	Material	PFC/10^6 of lymphoid cells — days after immunization												
				2	3	4	5	6	7	8	9	10	13	17	22	25
365	follicular	i.v.	blood	14.0	53.0	13.5	8.3	3.3	1.5	3.0	6.6	0	0	0	0+	0
			mucus	26.6	28.5	22.8	4.5	3.0	3.3	2.8	6.0	0	0	0	0+	0
			milk	0	8.9	11.1	4.2	6.6	3.9	0	0	0	0	0	0+	0
11	luteal	i.v.	blood	0	20.0	10.3	6.6	—	—	—	0	0	0	0+	0	0
			mucus	0	7.3	0	9.0	—	—	—	0	0	0	0+	30.4	0
			milk	0	3.1	0	0	0	—	—	—	0	0	0+	0	0
636	follicular	i.u.	blood	0	4.4	5.0	4.3	0.8	0	—	—	0	0	0+	0	0
111			mucus	0	26.7	18.3	10.5	9.5	8.3	—	—	9.2	0	0+	6.5	2.2
			milk	0	1.0	2.6	2.6	0	0	—	—	0	0	0+	0	0
28	luteal	i.u.	blood	0	6.9	4.0	10.8	6.7	0.7	1.3	3.3	0	0	0+	0	0
6			mucus	0	25.0	25.4	22.0	6.5	10.7	12.8	10.2	22.2	40.0	15.4+	9.0	8.5
			milk	0	8.5	—	3.0	1.0	0.9	0	0	0	0	0+	0	0

+ — oestrus

Table 2

Haemolysin titres in cows immunized with sheep red blood cells in various phases of the oestrous cycle

No. of cow	Phase of oestrous cycle	Route of antigen administration	Material	Days after immunization												
				2	3	4	5	6	7	10	13	17	22	29	35	51
365	follicular	i.v.	blood	40	160	80	160	160	160	10	10	40	10	1280	1280	80
			mucus	—	—	—	—	—	—	1280	1280	1280	1280	640	40	0
			milk	0	0	0	0	0	0	0	0	0	0	0	20	10
11	luteal	i.v.	blood	—	—	—	40	160	10	160	20	40	320	160	80	80
			mucus	—	—	—	0	0	0	0	0	80	10	20	40	0
			milk	—	—	0	0	0	0	0	0	0	10	10	0	0
636	follicular	i.u.	blood	—	—	—	45	15	30	25	15	10	30	25	15	10
111			mucus	0	0	—	0	0	5	0	5	20	45	25	0	10
			milk	0	0	5	0	0	0	0	0	0	0	0	0	0
28	luteal	i.u.	blood	10	20	50	20	20	20	10	5	15	10	90	50	40
6			mucus	—	—	—	—	—	—	15	40	25	20	10	0	0
			milk	0	0	0	0	0	0	0	0	0	0	0	0	0

ne administration of the antigen, the PFC number was higher in cows immunized in the luteal phase of the cycle (Table 1).

Serum haemagglutinin and haemolysin titres in cows immunized i.v. were, in general, higher when the cow was injected in the follicular phase. The animal displayed also a high level of cervical mucus antibodies (Tables 2 and 3).

Likewise, higher haemagglutinin titres in the cervical mucus were found in cows immunized in the follicular phase of the cycle. Antibodies, mainly haemolysins, were present also in milk both after intravenous and intrauterine administration of the antigen.

Discussion

The results reported in this study concerning the higher antibody titres in cows immunized in the follicular phase of the cycle than in that immunized in the luteal phase are in line with the observations of some authors (10) who obtained similar effects in mice, and are inconvenient with findings of those who demonstrated immunosuppresive action of oestrogens (8). Šjivič and Warr (9) suggest that the controversial results may result from different schedules of immunization and individual and species differences of the animals examined.

It was found that the cow immunized i.v. in the follicular phase i.e. in the oestrus, developed PFC a day earlier and by day 3 their number was twofold higher than that in the cow immunized in the luteal phase, i.e. at 12 days after the oestrus. Instead, the PFC response measured in the cervical mucus was more intense in cows injected i.u. in the progesterone phase than that in cows immunized in the oestrogen phase. Šljivič and Warr (8) noted a reduced splenic PFC response in mice treated with stilboestrol. The authors claim that the depressive action of stilboestrol might be mediated through the reticuloendothelial system (RES) stimulation leading to altered antigen distribution. They noticed also that it appears to be easier to demonstrate stilboestrol induced depression in terms of the PFC rather than serum anitbody titres.

It is of interest to note that two "peaks" of the PFC response were observed in cows immunized i.u. in the progesterone phase. The first "peak" occurred between 3 and 5 days after administration of the antigen, i.e. between 7 and 9 days of the oestrous cycle, whereas the other one between 10 and 13 days after immunization, i.e. between 14 and 17 days of the cycle. It would appear that this phenomenon results from an increase of blood oestrogen content which takes place between 10 and 13 days of the cycle and during the oestrus (12). The elevation of blood oestrogen level causes at first the reduction and afterwards a rapid increase in the number of PFC. This resembles the rebound effect which is observed after suppression of the oestrous cycle by gestagens and which is based on the appearance of intensive heat symptoms after an artificially produced anoestrus.

Table 3

Haemagglutinin titres in cows immunized with sheep red blood cells in various phases of the oestrous cycle

No. of cow	Phase of oestrous cycle	Route of antigen administration	Material	Days after immunization												
				2	3	4	5	6	7	10	13	17	22	29	35	51
365	follicular	i.v.	blood	40	40	40	160	40	80	20	20	20	20	20	40	20
			mucus	—	—	—	—	—	—	10	10	10	10	10	10	40
			milk	20	40	40	160	80	40	40	40	40	20	80	40	20
11	luteal	i.v.	blood	—	—	—	80	20	20	10	10	40	40	40	20	10
			mucus	—	—	—	0	10	0	0	0	0	20	20	10	80
			milk	—	—	40	20	40	80	80	40	20	10	10	10	0
636	follicular	i.u.	blood	—	—	—	10	10	10	15	15	10	15	15	40	10
111			mucus	20	40	—	15	90	60	30	30	30	25	330	330	330
			milk	5	60	25	40	30	40	30	30	20	15	10	0	5
28	luteal	i.u.	blood	40	40	30	20	40	40	5	5	10	10	25	15	20
6			mucus	—	—	—	—	—	—	10	15	10	5	0	0	5
			milk	25	30	60	60	60	40	40	20	30	15	30	45	45

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