glutamate-ergic synaptic inflow into the neuronal matrix, which provides for an anxiolytic effect in the model [3] where only the motivation of fear dominates (Table 1).

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MICROBIOLOGY AND IMMUNOLOGY

Neutrophil Chemiluminescence in Active Cytomegaloviral Infection

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Cytomegaloviral (CMV) infection is one of the most common viral infections occurring in the antenatal and postnatal period. The infection is diagnosed in 0.4-2.3% of all newborns [3,5], although the incidence differs in various populations. Harmless for the mother, in the fetus CMV infection may cause developmental defects and other grave complications which most frequently manifest themselves after birth in symptoms of central nervous system involvement, namely delayed mental development, microcephaly, blindness, deafness, epilepsy, muscular weakness, etc. [2,11]. The results of numerous studies on the course and outcome of CMV infection in pregnant women indicate a possible contribution of the relationships between CMV and the host immunity system to the pathogenesis of cytomegaly. An abundance of paper devoted to this problem have not yet got to

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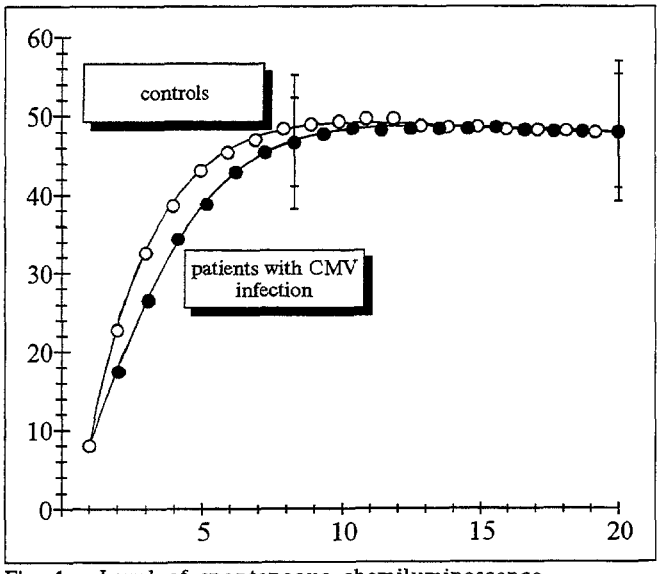

Fig. 1. Level of spontaneous chemiluminescence.

the bottom of it, and the role of granulocytes in antiviral immunity is virtually unknown, the available reports being contradictory [7,9]. Methods based on cellular chemiluminescence measurements open up new vistas for studies of neutrophil functional activity, because cellular chemiluminescence is closely related to oxygen metabolism or, to be more precise, to the so-called nonmitochondrial respiration, which, though not a survival system, forms the polymorphonuclear leukocyte (PMNL) biocidal activity metabolism [1, 2]. It is known that the phagocytosis process is not associated with the production of active oxygen forms and, hence, with chemiluminescence [2]. Oxygen is produced in the course of the biooxidation of phagolysosome contents and seems to be indirectly indicative of neutrophil phagocytic activity.

The present research was aimed at elucidating the relationship between patients' CMV infection and leukocyte phagocytic activity.

MATERIALS AND METHODS

Group 1 consisted of 10 nonpregnant women aged 25 to 36 (mean age 28.4±2.36) with an active CMV infection. The diagnosis was verified in all patients by the detection of viral DNA in the cells isolated from the urine and cervical mucus by dot bolt DNA hybridization and by the detection of specific IgM antibodies to CMV in the blood sera by enzyme immunoassay with commercial monoclonal antibody kits (Abbott, USA). Group 2 (controls) consisted of 10 women aged 23 to 35 (mean age 27.5±2.67) with normal reproductive function. Specific anti-CMV antibodies of class IgG were detected in the blood sera of all the women, this being evidence of a previous CMV infection. Both groups were matched for age, ethnic origin, physical development, socioeconomic and familial status, and types of genital and extragenital diseases. None of the patients presented with signs of exacerbations of any particular chronic genital or extragenital disease or of an acute infection at the moment of examination. Blood (4 ml) was collected in the middle of the second phase of the cycle in the morning on an empty stomach in tubes with 400 U heparin. The heparin-treated blood was diluted with 3% gelatin solution in a 1:1.5 ratio, thoroughly mixed, and incubated at 37°C for 30 min. Leukoconcentrate was collected, washed, and resuspended in medium 199, then layered onto a two-level Ficoll-Verografin gradient (specific densities 1.077 and 1.114 g/ml) and centrifuged for 30 min on a K23 centrifuge (Germany) at 2000 rpm. Granulocytes collected in the lower interphase were washed twice and resuspended in phosphate buffer solution (0.005 M, pH 7.4). The cells were counted in a Goryaev chamber, and their viability was assessed by 0.1% trypan blue staining. Luminol-dependent chemiluminescence was measured with an LKB 1251 Wallac luminometer using designated programs. For this purpose cooled phagocyte fractions (0.5×10^6 cells) and luminol (10^{-7}) were embedded in two thermostat cuvettes. Spontaneous luminol-dependent phagocyte chemiluminescence was measured for 20 min at 37°C. The maximal value (A_{sp}) and time needed to attain it (T_{sp}) were assessed. Then 1 μg/ml of human serum-opsonized zymosan (OZ) was added to one cuvette and 25 μg/ml phorbolmyristacetate (PMA) to the other. Induced chemiluminescence of phagocytes was measured for 20 min at 37°C. Its maximal value (A_{ind}), time needed to attain the maximal

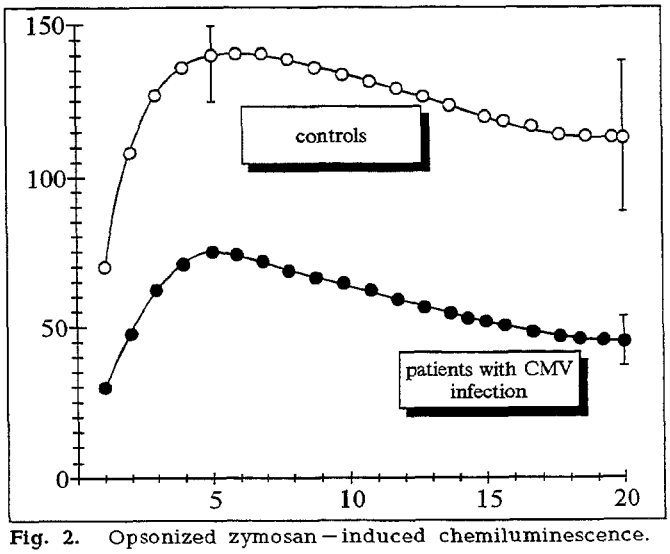

Fig. 2. Opsonized zymosan–induced chemiluminescence.

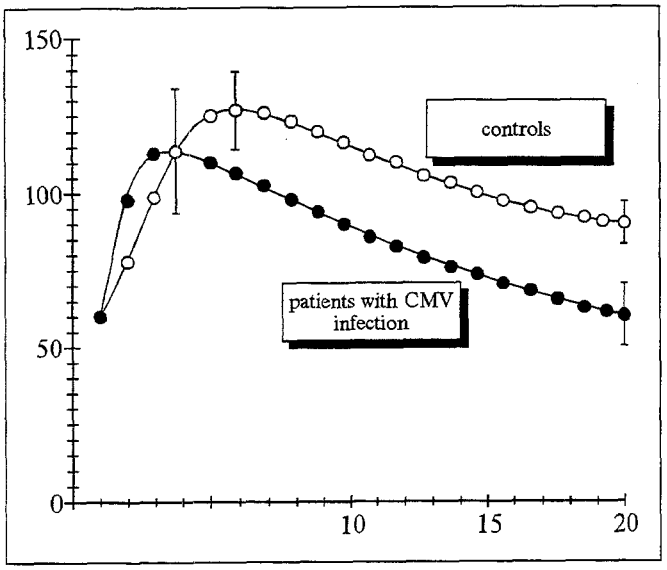

Fig. 3. PMA-induced chemiluminescence.

value (T_{ind}), and the stimulation coefficient (K_{st}), estimated as A_{ind}/A_{sp}, were assessed. Student's *t* test was used to process the results.

RESULTS

The results are presented in Table 1. The time course of the process is shown in Figs. 1-3. The data show no statistically significant differences between the two groups of women intact neutrophil chemiluminescence as regards both the maximal values of luminescence and the time needed to attain these values. Luminol-mediated chemiluminescence reached its peak at the 6th-7th min after incubation started and persisted thus till the end of the observation. Such a time course of the process was characteristic of both groups of women. Addition of OZ to the incubation medium enhanced chemiluminescence in both series of experiments, although in group 1 the stimulation coefficient was 1.63±0.226, whereas in the controls it was 2.94±0.326 (p_t<0.05).

The maximal chemiluminescence value was 50% lower in group 1 patients as compared to group 2: 76.6±21.34 and 145.5±22.27, respectively (p_t<0.05). Nevertheless, the time course of the process and the period needed to attain the maximal chemiluminescence coincided in the two groups. Cell incubation in the presence of PMA resulted in a sharp chemiluminescence increase: 2.45-fold in group 1 and 3.07-fold in group 2.

A reduced level of neutrophil luminol-mediated chemiluminescence in response to stimulation with OZ was revealed by other scintists *in vitro* with viruses belonging to different families [6-8]. However, the authors claim that the mechanisms underlying this phenomenon are specific for each of the viruses studied. Our results permit us assume that low phagocytosis activity is responsible for weak chemiluminescence of neutrophils in patients with CMV infection in response to OZ stimulation, because the neutrophils retain the capacity to respond by enhanced chemiluminescence to nonspecific activation of metabolic processes induced by PMA. Some authorities report that CMV can modulate the level of FC receptors to IgG on the cell surface [13], which seems to underlie the observed phenomenon because their participation in phagocytosis is universally asknowledged [4,10].

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TABLE 1. Peripheral Blood Neutrophil Chemiluminescence

Parameter	Group 1, n=10	Group 2, n=10	p_t<0.05
A_{sp}, mV	47.4±12.97	48.2±5.56	–
T_{sp}, min	6.4±0.70	6.3±1.04	–
A_{ind}OZ, mV	76.6±21.34	145.5±22.27	+
T_{ind}OZ, min	5.2±0.35	6.1±0.67	–
K_{st}OZ	1.63±0.226	2.94±0.326	+
A_{ind}PMA, mV	116.4±25.97	135.8±15.55	–
T_{ind}PMA, min	4.7±0.82	6.9±0.55	–
K_{st}PMA	2.45±0.478	3.07±0.312	–