TEMPERATURE INFLUENCE ON STIMULATED PMN RESPIRATORY BURST

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INTRODUCTION

Body temperature in humans, as in all mammals, is dependent on exoergonic biochemical reactions which produce the heat diffused by the cardiovascular system. Body temperature can also be influenced by environmental temperature and by physical exercise.

Thermoregulation includes a series of physiological mechanisms regulated by the nervous system which keep body temperature constant, by opposing decrease and increase in heat. However, temperature varies in the different parts of the body. It tends to diminish moving from internal organs towards the epidermis, mainly in the epidermis of extremities. This range in temperature may influence the development and clinical manifestations of infectious, metabolic and immunologic diseases.

All living organisms, including pathogenic agents, have their own ecologic niche, characterized by a particular temperature level. M. Laepre and M. Ulcerans grow between 27 and 30° C while thermal increase and decrease inhibit the growth of bacteria. For this reason the above mentioned mycobacteria preferably infect the epidermis or mucosas which are "colder" areas than internal organs (2).

The solubility of monosodic urate (MSU) crystals characterizing gout, drastically diminishes under 25°C: these temperatures, which favour crystal precipitation, are registered in the early morning hours in the I metatarsal-phalangeal joint which is the typical site of acute gout attack (3).

Antigen-antibody reactions are deeply influenced by temperature as well. In some autoimmune diseases involving small and medium size blood vessels, such as cryoglobulinemia, circulating immunoglobulins reversibly precipitate at temperatures lower than 37°C (4). IgM, called cold agglutinins, are detected in autoimmune hemolytic diseases and cause agglutination of red cells solely at blood temperatures lower than 28-32°C. Erythrocytes are also destroyed when areas of the body reach a temperature lower than 20°C, which can easily happen on the skin. Under similar circumstances, therefore, there is the fixation of the first fragments of the complement (5).

On the other hand, in several pathologic processes there is a local increase (for example at the inflammatory site) or a general increase (febrile state) of body temperature: this is the response of the organism to disease (6,7). In general, this febrile response is beneficial to the individual and when it is prevented, particularly in some infectious diseases, the prognosis can be very poor.

The processes so far described, may possibly be explained in the capability of body temperature to affect various cellular functions and for this reason the influence of thermal variations on white blood cells is of prime importance. Leucocyte functions are influenced by temperature in various respects: for example, leucocyte adherence to endothelial cells, recruitment and aggregation of granulocytes and phagocytic activity. Effects of temperature have also been detected on integrin adherence, leucocytosis and antibody and cytokine production (8-16). The observations reported in the literature have led us to consider granulocyte response to temperature in relation to the production of oxygen free radicals (OFR) which have a key role in the mechanism of inflammation not only for non-specific defences against infectious agents and in the autoimmunity and disregulation of pathogenic mechanisms.

MATERIALS AND METHODS

In our study we evaluated by chemiluminescence detection system (CL) (17) the variation in the production of oxygen free radicals by human

polymorphonuclear cells stimulated with opsonized Zymosan (OZ), phorbol myristate acetate (PMA), formyl-methionyl-leucyl-phenylalanine (FMLP), opsonized monosodium urate (MSU) crystals, in different thermal conditions (26, 37, 39, 40 and 42°C).

Briefly, PMNs were stimulated with Zymosan (10 mg/ml phosphate buffer saline-PBS-without Ca^{2+} and Mg^{2+}, Sigma) opsonized according to Bellavite's model (18), FMLP (6 μM in dimethyl sulphoxide, Sigma), PMA (9.6 μM in dimethyl sulphoxide, Sigma) and MSU crystal (10 mg/ml PBS, Sigma) were opsonized according to Bellavite.

Human PMNs were obtained from heparinized venous blood by polymorphoprep (Nycomed) sedimentation and erythrocyte lysis with NH_4Cl (0.83%). Neutrophils were suspended in PBS at a final concentration of $1x10^6$ cells/ml. Final cell suspension contained a minimun of 90% neutrophils. Viability of the neutrophils was greater than 96% as determined by trypan blue exclusion.

The technique of luminol dependent CL was used as indicator of OFR release by stimulated PMNs. The reaction mixture contained 100 μl PMN suspension, 100 μl luminol solution (2 mg in 10 ml of NaOH 0.1 M, subsequently diluted 1:10 with PBS, Sigma) and 10 μl of each stimulator. The preparations were placed in a chemiluminometer (Berthold Multi-biolumat LB 9505C) at 26, 37, 39, 40 and 42°C; the kinetics of reaction was recorded for 40 minutes. Each experiment was repeated 6 times for each concentration of the tested substances, for an adequate statistical analysis. Statistical analysis was performed utilizing the matched-data Wilcoxon test.

RESULTS

In the bar chart (fig. 1) statistical significances are reported for the differences in the production of OFR by PMNs diversely stimulated at 26-37-39-40-42°C temperature. In each condition significances were calculated considering the production of OFR at 37°C.

Table 1 shows mean values and relative standard deviations obtained at different temperatures with the various stimulators utilized.

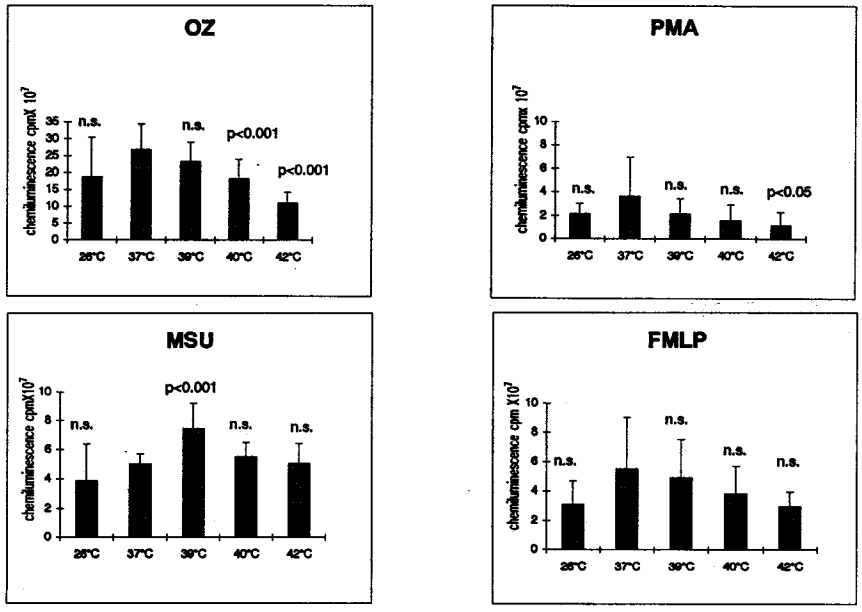

Fig. 1 - CL responses by PMNs stimulated with OZ, PMA, MSU crystals and FMLP at different temperatures. In each condition significances were calculated considering the CL response at 37°C.

Tab. 1 - Mean values and relative standard deviations of chemiluminescence response obtained by PMNs diversely stimulated at 26-37-39-40-42°C.

	cpm- 26°C ±D.S.	cpm - 37°C±D.S.	cpm - 39°C±D.S.	cpm -40°C±D.S.	cpm-42°C±D.S.
ZYMOSAN	1.87±1.17 (10^8)	2.67±0.76 (10^8)	2.33±0.57 (10^8)	1.82±0.58 (10^8)	1.1±0.31 (10^8)
MSU	3.86±2..5 (10^7)	4.99±0.7 (10^7)	7.42±1.8 (10^7)	5.5±0.97 (10^7)	5.02±1.37 (10^7)
PMA	2.14±0.88 (10^7)	3.63±3.37 (10^7)	2.15±1.3 (10^7)	1.58±1.36 (10^7)	1.16±1.1 (10^7)
FMLP	3.12±1.57(10^7)	5.52±3.5 (10^7)	4.94±2.55(10^7)	3.84±1.84(10^7)	2.98±1.0 (10^7)

It can be noticed that OZ, PMA, FMLP induced a higher response at 37°C as compared to the other temperatures. In particular, a significant statistical decrease was observed in the chemiluminescence values at

temperatures of 40-42°C ($p<0.001$) and 42°C ($p<0.05$) in the presence of OZ and PMA respectively.
In contrast, PMNs stimulated with MSU crystals produced a higher CL response at 39°C, so that the detected increase became statistically significant at the temperature of 37°C ($p<0.001$).

DISCUSSION

Our interest in the evaluation of the influence of temperature variations on granulocyte functions relevant for non-specific defences of the human body and of inflammation phenomena, has prompted us to examine the effect on the "respiratory burst". The results of the chemiluminescence assay showed significant variations in the granulocyte function at temperatures which are known to be reached by the human body in pathological conditions. PMN response induced by OZ, PMA, FMLP cell stimulators reached maximum at 37°C.
In particular, OFR production from PMN stimulated with OZ, PMA, FMLP was higher at 37°C than at 26, 39, 40, 42°C ($p<0.001$ OZ stimulated PMN at 40-42°C; $p<0.05$ PMA stimulated PMN at 42°C. Significantly different from 37°C value).
PMN stimulation with MSU crystals, in contrast with what described so far, caused a significant increase of the production of reactive species of oxygen at the temperature of 39°C compared to 37°C value ($p<0.001$). This different behaviour cannot be accounted for by the cellular response solely in relation to thermal variation, but it is clear that the specific cellular response to microcrystals and their chemical-physical variations with the changes of temperature also play an important role. Thermal increases and exposure to OFR may cooperate in inducing a decrease in the size and number of crystals, making them inactive. Since the size of crystals can modify the entity of the production of the oxygen reactive species, their progressive reduction, may explain the ending of the inflammatory process with the consequent resolution of the acute gout attack (19).

Our aim is to carry out further studies in this field in order to evaluate the behaviour of other crystals.

Temperature variations, as reported in our paper, can affect a major component of the non-specific defenses of the human body and of the pathogenesis of inflammation.

These results allow us to consider that temperature may influence flogistic joint localizations of rheumatic diseases. Furthermore, these studies can offer references for a more rational utilization of administration (thermotherapy) and subtraction (criotherapy) of heat at local sites as well as new perspectives in the understanding of advantages and disadvantages of general hyperthermia in pathological and dysregulation conditions of the human body.

Body temperature can modulate the pathogenesis of infectious, metabolic and autoimmune diseases. This effect has been attributed to several hypothesized mechanisms. Body temperature could play an important role in influencing some cellular functions of human white blood cells. In this work we examined the temperature effect on the respiratory burst in human neutrophils. Human polymorphonuclear leucocytes (PMN) were obtained from heparinized venous blood by dextran sedimentation and erythrocyte lysis with NH_4Cl (0.87%). Granulocytes were stimulated with opsonized zymosan (OZ), formyl-methionyl-leucyl-phenylalanine (FMLP), phorbol myristate acetate (PMA), and monosodium urate (MSU) crystals at different temperatures (26, 37, 39, 40, 42°C). The technique of luminol dependent chemiluminescence (CL) was used as indicator of oxygen free radicals (OFR) release by stimulated cells. OFR production from PMN stimulated with OZ, PMA, FMLP was higher at 37°C than at 26, 39, 40, 42°C ($p<0.001$ OZ stimulated PMN at 40-42°C; $p<0.05$ PMA stimulated PMN at 42°C. Significantly different from 37°C value). OFR release from PMN stimulated with MSU crystals was significantly increased at 39°C compared to 37°C value ($p<0.001$). This effect could not only be attributed to temperature influence on neutrophil activity. The specific polymorphonuclear

leukocyte response to the microcrystals and the temperature influence on chemical and physical characteristics of the crystals may play an important role. We are now studying the temperature effect on activity of PMN exposed to others crystals.

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