100 Smith, C. W., Jafary-Asl, A. H., Choy, R. Y. S., and Monro, J. A., The emission of low intensity electromagnetic radiation from multiple allergy patients and other biological systems, in: Photon Emission from Biological Systems, pp. 110–126. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, Singapore 1987.
101 Sweeney, B. M., The loss of the circadian rhythm in photosynthesis in an old strain of *Gonyaulax polyedra*. Pl. Physiol. *80* (1986) 978–981.
102 Swinbanks, D., Body light points to health. Nature *324* (1986) 203.
103 Tombesi, P., and Pike E. R. (Eds), Squeezed and Nonclassical Light. Plenum Press, New York and London 1989.
104 Tryka, S., and Koper, R., Luminescence of cereal grain subjected to the effect of mechanical loads, in: Photon Emission from Biological Systems, pp. 248–254. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, Singapore 1987.
105 van Wijk, R., and van Aken, H., Spontaneous and light-induced photon emission by rat hepatocytes and by hepatoma cells, in: Recent Advances in Biophoton Research and its Applications, pp. 207–229. Eds F. A. Popp, K. H. Li and Q. Gu. World Scientific, Singapore, New Jersey, London, Hong Kong 1992.
106 van Wijk, R., Regulatory aspects of low intensity photon emission. Experientia *44* (1988) 586–593.
107 Veselowskii, V. A., Sekamova, Y. N., and Tarusov, B. N., Mechanism of ultraweak spontaneous luminescence of organisms. Biophysics *8* (1963) 147.
108 Vigny, P., and Duquesne, M., On the fluorescence properties of nucleotides and polynucleotides at soom temperature, in: Excited States of Biological Molecules, pp. 167–177. Ed. J. B. Birks. J. Wiley, London 1976.
109 Wolkowski, Z. W., Hierarchical aspects of synergy and coherence in biological systems, in: Photon Emission from Biological Systems, pp. 22–50. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, Singapore 1987.
110 Yanbastiev, M. I., On some applied-medical aspects of biophotons, in: Photon Emission from Biological Systems, pp. 184–198. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, Singapore 1987.
111 Yoda, B., Goto, Y., Saeki, A., and Inaba, H., Chemiluminescence of smoker's blood and its possible relationship to cigarette smoke components, in: Photon Emission from Biological Systems, pp. 199–210. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, Singapore 1987.
112 Yuen, H. P., Nonclassical light, in: Photons and Quantum Fluctuations, pp. 1–9. Eds E. R. Pike and H. Walther. Adam Hilger, Bristol and Philadelphia 1988.
113 Zevenboom, W., and Mur, L. C., Growth and photosynthetic response of the cyanobacterium *Microcystis aeruginosa* in relation to photoperiodicity and irradiance. Archs Microbiol. *139* (1984) 232–239.
114 Zhuravlev, A. I., Tsvylev, O. P., and Zubkova, S. M., Spontaneous endogeneous ultraweak luminescence of the mitochondria of the rat liver in conditions of normal metabolism. Biophysics *18* (1973) 1101.

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Photon emission of phagocytes in relation to stress and disease

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Abstract. Phagocytes, the first-line cells of the body's defence mechanisms against invading pathogens, kill microorganisms by means of lysosomal degradative enzymes and highly toxic reactive oxygen intermediates. The reactive oxygen compounds are produced, in a process called the 'respiratory burst', by the NADPH oxidase complex in plasma membranes, and by myeloperoxidase in phagolysosomes after degranulation. These processes generate electronically excited states which, on relaxation, emit photons, giving rise to phagocyte chemiluminescence (CL). This paper describes the conditions for the measurement of CL, and reviews the activity of phagocytes from individuals undergoing stress or disease. The capability of phagocytes to emit photons reflects remarkably well the pathophysiological state of the host. In many cases even the magnitude of the stress, the presence of a pathogen in the body, or the activity of the disease can be estimated. Physiological changes, e.g. in the reproductive cycle, can also be predicted.
Key words. Chemiluminescence; phagocyte; stress; disease.

Introduction

Phagocytosing leukocytes constitute the first line of the body's defence mechanism against invading microbial pathogens. Neutrophils (polymorphonuclear leukocytes, PMNL) are the first cells to invade a site of inflammation following an infection. In an inflammatory response the neutrophils are followed later by activated monocytes, macrophages and – especially in the case of parasitic infection – also by eosinophils.

Phagocytes kill microorganisms by means of lysosomal degradative enzymes, such as proteases, and highly toxic reactive oxygen metabolites. Killing processes can take place inside the cell in phagolysosomes as well as outside the phagocyte.

In a process called the 'respiratory burst' activated phagocytes reduce molecular oxygen to superoxide via a special electron transport system (NADPH-oxidase). Superoxide radicals form hydrogen peroxide in a dismutase reaction catalyzed by the superoxide dismutase enzyme (SOD). Hydrogen peroxide serves as a substrate for the myeloperoxidase (MPO) reaction, in which a variety of highly toxic metabolites, including hypochlorite, are generated. These processes produce electronically excited

states which, on relaxation to the ground state, emit photons. This emission is referred to as phagocyte chemiluminescence (CL).

Lucigenin and luminol amplify the CL emission by factors of 10^2-10^3 and 10^3-10^4, respectively. Lucigenin has a high specifity for the superoxide radical, and thus lucigenin CL reflects the activity of the NADPH-oxidase complex, whereas luminol CL is dependent on MPO activity.

Neutrophils, eosinophils and monocytes have both NADPH-oxidase and MPO activity, and when activated they generate both lucigenin- and luminol-enhanced CL. Macrophages, when primed or activated, are able to generate lucigenin CL, but in the course of maturation the MPO content decreases and thus mature macrophages have a diminished luminol-enhanced CL response.

The commonly used activators include opsonized or unopsonized zymosan, a chemotactic peptide n-formyl-methionine-leucyl-phenylalanine (fMLP), immune complexes, the membrane perturber phorbol myristate acetate (PMA), and calcium ionophore A23187. Zymosan is a cell wall preparation of *Saccharomyces cerevisiae* containing glucan and mannan, which are recognized by the complement receptor 3 complex (CR3). CR3 also recognizes C3bi and possibly fibrinogen. The binding is dependent on the divalent cations calcium and magnesium. In the opsonization process zymosan attaches to complement compounds and immunoglobulins (Ig). Opsonized zymosan is recognized partly by CR3, partly by CR1 which binds to C3b, and partly by FcγRII and FcγRIII receptors which bind to the Fc portion of the IgG molecules attached on zymosan particles. The FcγRI receptor apparently mediates the antibody-dependent cell-mediated cytotoxicity (ADCC) reaction. Expression of receptors on cell membranes, changes in their functional capacity, signal transduction, phagocytosis, and degranulation all participate in the CL response. Defects in these processes attenuate the response.

Recently, attention has been focused on the hazards of and the damage caused by phagocyte infiltration into tissues and by release of reactive oxygen intermediates. The myocardium is infiltrated within minutes from the onset of infarction, the kidneys in certain types of glomerulonephritis, and the lungs in several pathological conditions. The hyper- or hypoactivity of phagocytes is a decisive factor in the pathogenesis of many diseases like rheumatoid arthritis. The next sections describe the measurement of phagocyte CL and the beneficial and detrimental consequences of the production of reactive oxygen species by phagocytes.

Method

Phagocytic cells are generally isolated from blood treated with anticoagulants or from other biological fluids using standard gradient centrifugation methods. On many occasions the buffy coat obtained from blood after erythrocyte sedimentation can be used as a source of phagocytes without further separation.

Phagocytic cell activities can also be measured in ex vivo state simply by diluting whole blood or other body fluids enough to get rid of the inhibitory amounts of plasma and red cells. If the blood samples are not dilute enough, the opsonins in the plasma can interfere, especially when unopsonized particles are used as stimulants. It should be noted here that ex vivo cells are not necessarily in the same functional state as the cells after isolation steps where activation processes may take place. Cooling and rewarming should be avoided because of altered receptor expression.

It has been claimed that blood cells other than phagocytes (lymphocytes, NK cells) were also able to emit CL, but in all cases investigated so far the contaminating phagocytes have been shown to be the actual source. B-lymphocytes transformed by the Epstein-Barr virus might be an exception.

Researchers nowadays seldom carry out phagocyte CL tests without amplifiers: luminol and lucigenin are generally used. Lucigenin reacts with superoxide anion and needs to be reduced to become luminescent. It is therefore considered to be dependent on NADPH oxidase activity. Luminol, on the other hand, has been shown to be oxidized in the myeloperoxidase reaction. When using luminol in the millimolar range one needs less than a thousand phagocytic cells (as in the case in whole blood tests) to get reliable signals. The number of isolated cells used in routine tests varies, generally around 10^5. If adhesion is not being specially studied, gelatin (or other proteins) should be used to prevent aggregation and the adhesion of the cells to the walls of the measuring vials. Hank's balanced salt solution is probably the most frequently used buffer. If other buffers are used one should pay attention especially to their content of divalent cations.

Liquid scintillation counters are not recommended as measuring devices because of poor temperature control. Modern luminometers with strict temperature controls, multiple sample capabilities (up to 96 in microtiter plate readers), and computerized data processing are the instruments of choice.

Defects in phagocyte functions

An individual suffering from recurrent infections, which are often severe and may eventually be fatal, may have a defect (often one of genetic origin) in one of the crucial functions of phagocytic cells.

Chronic granulomatous disease (CGD) is a rare disorder in which the patients suffer from severe recurrent infections with bacteria and fungi, owing to an inability of their phagocytes to kill catalase-positive microorganisms. This is caused by the failure of CGD leukocytes to produce sufficient amounts of superoxide and hydrogen peroxide during phagocytosis. The defect has been

shown to be located in the b-type cytochrome of the NADPH-oxidase complex. Either a deficiency of the complex or a defect in the redox reactions with cytochrome b is the reason [132]. Phagocytes of CGD patients were not able to emit CL [7, 8, 29, 36, 92, 132] except in those cases where hydrogen peroxide-generating microbes (e.g. *Streptococcus pneumoniae*) had been ingested [8], which indicated that the defect in CGD is negated by peroxide generated by microbes.

Subjects with myeloperoxidase (MPO) deficiency have rarely been reported. Although MPO in the presence of hydrogen peroxide and halide constitutes a potent bactericidal system, which is also effective against fungi, viruses, mycoplasma, and mammalian tumour cells, MPO-deficient subjects only rarely have severe infections, mainly candidiasis. Superoxide generation by MPO-deficient neutrophils was augmented [70]. The CL response from these cells in the absence of amplifiers was reduced compared to that of control cells but it was still well measurable [70, 109] which indicates that the native CL signal from phagocytosis is dependent on both NADPH oxidase and MPO. MPO-deficient cells emitted practically no luminol-amplified CL, confirming the dependence of luminol CL on MPO. On the other hand, lucigenin-amplified CL was augmented, confirming the dependence of lucigenin CL on superoxide generation by NADPH oxidase.

Defective degranulation of MPO was suspected to be the reason for recurrent, superficial abscesses caused by *Staphylococcus aureus* in one patient. Neutrophils showed an impaired luminol-dependent CL emission in response to stimulation by either latex beads or fMLP [41]. It was suggested that the defect represented abnormal microtubule microfilament function, as has also been suggested with the Chediak-Higashi Syndrome.

Leukocyte adhesion deficiency (LAD) is characterized by defective expression of leukocyte adhesion glycoproteins CD11a/CD18 (LFA-1), CD11b/CD18 (CR3), and CD11c/CD18 (p150,95). The patients have recurrent severe bacterial infections. The phagocytic cells from these patients showed no marked CL response when stimulated with zymosan in the presence of either luminol or lucigenin. On the other hand, they did show a CL response with both amplifiers when stimulated with IgG_{2a}-coated sheep red cells. Control PMNLs did not show any CL response to this stimulant, although monocytes from both patients and controls gave CL responses of similar magnitude. These results suggest that in LAD patients FcγI receptors on PMNLs were responsible for CL generation [85].

Infections

PMNLs represent cornerstones of the host's antimicrobial defence system, and several disease syndromes characterized by chronic or recurrent infections have been related to defects in PMNL function. Alterations in PMNL function seem to occur during microbial infections in patients with normal defence mechanisms. The morphological changes are also well established. It has been suggested that the blood of patients with acute infections might contain mixtures of normal, primed, and perhaps deactivated or exhausted PMNL.

Basal CL, as well as CL stimulated by opsonized zymosan, fMLP, and PMA, were generally increased in isolated PMNLs and in whole blood during acute bacterial infections [3, 17, 52, 54, 80, 82, 88, 97, 122, 126]. However, some patients had reduced PMNL function, and this reduction may contribute to a fatal outcome of the disease [122]. The enhancement of the CL activity was observed during the period of fever and the activity gradually declined during the postfebrile convalescence period [3, 82, 88, 97]. An important observation is that when rats were challenged with *Francisella tularensis*, immune rats showed lower CL activation than nonimmune rats. In contrast to nonimmune rats, fever was not detected in immune rats at the maximum PMNL activation. The activation period lasted only for a couple of days, whereas that of nonimmune rats continued for more than one week [88]. Thus the decreasing CL response may be attributed to inhibited dissemination or rapid clearance of bacteria. Indeed, we observed that in mice infected intravenously with *E. coli* the whole blood CL activity was at its highest one day after infection, and bacteria were abundant in the blood. On the other hand, on day 3 after the challenge the CL value had returned to the normal range and no bacteria could be detected in the blood (unpublished). The alteration in PMNL function must start before the onset of fever. This concept is supported by our observation that the temperature range of maximum CL activity rose by 2–3 °C in both febrile and non-febrile patients suffering from various kinds of respiratory tract infections [86]. The degree of pathogenicity of bacteria is reflected in the magnitude of phagocyte CL activity. We observed that in mastitis minor, pathogens caused only an increase in the number of CL-emitting cells in milk, whereas major pathogens also increased the activity of phagocytes [82], probably by priming. One reason for the increased CL activity in bacterial infections can also be the emergence of hyperactive subpopulations of neutrophils in the circulation [18, 74, 93].

Epidemiological evidence in humans supports the hypothesis that primary viral infections cause increased susceptibility to bacterial disease. Patients and animals with viral infections usually had reduced or normal CL activity in PMNLs, whole blood, or peritoneal macrophages when stimulated by zymosan, opsonized latex, or phorbol esters during the acute infection [1, 17, 43, 77, 88, 122, 126]. Animal models showed that the phagocyte CL activity remained in the normal range during the viremic period but thereafter declined below normal [1, 43, 88]. The depression of opsonized zymosan and PMA-stimulated human PMNL CL activity by influenza A virus was shown to be dependent on the haemagglutination activity of virus

pools[28,45], and the prepriming of the PMNLs overcame this dysfunction in vitro[4] as well as in vivo in mice in which the mortality was significantly reduced[90,110].

In summary, it seems that bacterial infections cause the increase in phagocyte activity by increasing the number of phagocytes, by priming the cells, or by causing hyperactive subpopulations to emerge. Priming by bacterial compounds makes the host's phagocytes more potent in resisting viral infections. On the other hand, viral infections disable the phagocytes for resistance to subsequent bacterial infections.

Stress

Individuals may encounter various stressful events during their life span. Bacterial infections are a major cause of morbidity in individuals under stress and may be fatal. Depressed individuals are also more susceptible to cancer. Chronic exposure to elevated levels of stress hormones, e.g. cortisol, may lead to immunosuppression.

Pre-term. Neonates, especially pre-term infants who require intensive care, suffer from an increased susceptibility to infections which rapidly become systemic. Numerous reports show that the CL activity of PMNLs stimulated with opsonized zymosan or PMA was significantly depressed in neonates[20,21,40,44,46,91,98,119,129]. Alveolar macrophages in neonatal pigs similarly had very low CL activity as compared to 7-day-old piglets or adult pigs[139]. Serious infections developed more frequently in infants with low PMNL CL activity during the first week of life than in infants with normal PMNL responses. Moreover, the mean peak CL activity did not differ before, during and after serious infections[40]. Other reports have also shown that, unlike older children and adults, neonates showed low PMNL CL activities during bacterial infections[21,46].

Trauma. Operative trauma caused a depression of PMNL CL activity[24,100] which was shown to be mainly due to the anesthesia[24]. In patients with major postoperative infection, CL response was depressed, but no such changes were seen in patients with minor postoperative infection[113]. Depressed activity of phagocytes, however, may not be the only cause of increased postoperative susceptibility to bacterial infections, since defects in the opsonic capacity of serum during anesthesia and surgery were also observed[89,101]. Other types of trauma led to an increase in the CL activity of PMNLs[9,102,111,116] except in burned patients with burn areas more than 35%. In addition to CL activities lower than those in the control, these patients also had impaired opsonic activity, and the incidence of sepsis was very high (85%). Furthermore, all the patients who did not survive had very low PMNL CL activity[116].

Exercise. Acute strenuous physical exertion is accompanied by physiological changes that are in some respects similar to those induced by bacterial infection: there is a substantial increase in circulating leukocytes, an elevation of body temperature, and an increase in concentrations of serum factors such as interleukin-1, α-interferon, and acute phase proteins. Trained athletes are, however, more susceptible to common infections than normal people. Their phagocyte functions are attenuated. Reports of the effects of acute exercise on phagocyte CL activity are controversial. Both an increase[99,121] and a decrease[72,136] of CL after single bout of exercise have been reported. A long period of regular intensive training led to suppression of phagocyte CL[121]. Our experiments with both humans and rats showed similar results depending on how strenuous and long-lasting exercise had been. Moderate exercise increased the CL activity but very hard exercise depressed it (unpublished). It is known that a prolonged or particularly large physical load inevitably leads to injuries in muscles, lungs and tendons, resulting in substantial phagocyte infiltration into these tissues. We believe that a single bout of strenuous exercise primes phagocytes, but hard physical stress leading to tissue injuries and leukocyte infiltration is reflected as decreased CL activity in peripheral blood, since the hyperactive phagocyte subpopulation is probably the first to leave the circulation.

Psychological factors. How psychological stress caused by stressful life events, or factors like self esteem and personal control, influence phagocyte functions, has to our knowledge been considered in only one report[95]. CL responses of neutrophils were decreased in panic disorder patients as well as during endogenous depression, but remained normal in schizophrenia, alcoholism and generalised anxiety. Suppression was corrected on clinical recovery.

Oxidative stress. A diet rich in polyunsaturated fatty acids (cod liver oil rich in eicosapentaenoic acid and docosahexaenoic acid) caused a decrease in the CL activity of phagocytes in healthy and arthritic humans[49,84] and in rats[79]. However, another fish oil, mackerel oil, with a four-fold higher content of arachidonic acid did not have the same inhibitory effect in rats. The analysis of fatty acid content of membrane phospholipids revealed that a cod liver oil diet caused a significant decrease in arachidonic acid in phospholipid while a mackerel oil diet did not. Vitamin E supplementation reduced the suppressive effect of a cod liver oil diet[79], suggesting that the oxidation products of polyunsaturated fatty acids play a role in the suppression. Experimental essential fatty acid deficiency in rats similarly suppressed the phagocyte CL activity[57,58]. Hypercholesterolemia is supposed to be a consequence of, for example, diets with a high saturated fatty acid content. Subjects with hypercholesterolemia had a slightly higher level of arachidonic acid in membrane phospholipids and significantly increased CL activity of isolated neutrophils[34]. A corn oil diet markedly increased the number of tumours per mouse compared with a beef tallow diet[94].

These results suggest that dietary factors leading to an altered arachidonic acid content in the membrane phos-

pholipids of phagocytes and consequently altered production of prostaglandins and leukotrienes have a crucial role in the regulation of the CL response.

Another type of oxidative stress is introduced by smoking. Cigarette smoke is known to contain reactive peroxy radicals. Whole blood of cigarette smokers produced more CL than that of non-smokers when stimulated with opsonized zymosan, PMA, or fMLP[10,11,63,106,107]. The number of cigarettes smoked per day, and lung function measured by spirometry, were shown to be in a good correlation with the leukocyte CL value in young smokers[106]. Passive smoking also primed neutrophils to emit increased amounts of CL[11].

Respiratory disorders

Sarcoidosis is a systemic disorder of unknown etiology which, in many patients, is associated with progressive pulmonary fibrosis. It is therefore not surprising that both circulating neutrophils and bronchoalveolar lavage cells from sarcoidosis patients were more active than those from controls in emitting basal CL and CL stimulated by opsonized zymosan or PMA[25,67,87,130].

Cystic fibrosis patients suffer from pulmonary *Pseudomonas* infections, but they have little difficulty in containing infections outside the respiratory tract. In patients PMNLs and monocytes from peripheral blood were activated[56,105,108], but unfortunately there are no reports concerning the activity of bronchoalveolar lavage cells in this disease. On the other hand, increased CL activities of alveolar cells have been detected in hypersensitivity pneumonitis[26], asthma[31], idiopathic pulmonary fibrosis[68], primary biliary cirrhosis-associated alveolitis[131], and endotoxin-induced lung injury in dogs[60]. In respiratory disorders air space cells seem generally to be activated. The activation however, is not only local; in many occasions systemic activation is evident.

Diabetes

It is generally believed that diabetes mellitus is associated with an increased susceptibility to infection or severity of infections. The presence of microvascular endothelial injuries in diabetic patients is well known and it has been suggested that phagocyte-mediated reactive oxygen intermediates are involved in damage to pancreatic islet cells in insulin-dependent disease.

The literature on CL supports these concepts. When stimulated with opsonized zymosan, both PMNLs[15] and monocytes[69] showed increased CL emission in patients as compared to controls. However, when zymosan was opsonized in autologous plasma the CL response was lower than in controls[133]. The increased neutrophil activity was correlated with circulating immune complexes and both parameters were related to the presence of microvascular complications[16]. The resting CL activity of isolated PMNLs from diabetic children was significantly higher than in controls[65]. The whole blood CL responses to soluble stimuli, but not to particulate stimuli, were enhanced in patients as compared to controls[37].

It seems that in diabetes the phagocytic cells are primed for production of reactive oxygen species but there are inhibitory compounds like circulating immune complexes in plasma which, by blocking the Fc receptors, may diminish the phagocytic capacity.

Arthritis

The inflammation of joints may be of a chronic recurrent type (rheumatoid arthritis) which is considered to be autoimmune in nature, or acute reactive arthritis preceded by an infection elsewhere in the body. It has been suggested that the presence of immune complexes in the synovial fluid leads to the inflammatory reaction. It could be thought that synovial fluid phagocytes and possibly peripheral blood phagocytes were primed for the enhanced production of reactive oxygen intermediates.

In fact, in rheumatoid patients the basal CL activity of synovial PMNLs (SFPMNL) was higher than that of peripheral blood cells (PBPMNL), but when stimulated with opsonized zymosan PBPMNLs showed higher activity[61]. This work also showed that when stimulated with heat-aggregated IgG, SFPMNLs gave much higher activity than PBPMNLs, but the opposite was found after preincubation with fMLP. While SFPMNL activities were lower than PBPMNL activities in patients, PBPMNL activities of patients did not differ significantly from PBPMNL activities of healthy controls[76]. An effect of the severity of the disease was seen in the CL response of SFPMNL induced by PMA. The response was significantly higher in patients with severe disease than in those with mild disease[76]. It is not only priming that may cause the increased production of reactive oxygen species in inflamed joints. When paired sera and synovial fluids from rheumatoid arthritis patients were incubated with control neutrophils, synovial fluids gave considerably higher CL responses than the paired serum specimens. In contrast, little or no response was found with paired sera and joint fluid taken from patients with gout, psoriasis, and osteoarthritis, or with sera from healthy donors. The active material found in the rheumatoid specimens was suggested to be particular types of immunoglobulin and rheuma factor complexes[55].

Thus, in arthritis the SFPMNLs seem to be primed and the synovial fluid contains compounds that evoke the SFPMNLs to produce considerable amounts of reactive oxygen intermediates. The activation of phagocytes seems not to be systemic.

Psoriasis

Psoriasis is a skin disease of unknown aetiology characterized by hyperactivity of keratinocytes leading to increased keratinization of the skin, giving it a scaly ap-

pearance. Peripheral blood monocytes and neutrophils have been shown to be more active in psoriasis patients than in controls when stimulated with opsonized zymosan, fMLP or PMA[32, 114, 120]. Exudate neutrophils from skin were primed both in psoriatic patients and in controls, showing no significant difference[33]. These results suggest that psoriasis is systemic in nature.

Inflammatory bowel disease

Neutrophils are absent from the normal intestine, but in inflammatory bowel disease they move efficiently from the blood across the mucosa to the intestinal lumen, mediating tissue damage.

The resting and the opsonized zymosan-stimulated CL responses were significantly increased in children who had the disease only mildly or were in remission[48]. Neutrophils from patients with Crohn's disease, but not from those with ulcerative colitis, were found to have significantly increased numbers of receptors for fMLP. The receptor number had a linear positive correlation with peak CL response to fMLP. Drug treatment and disease activity had no effect on these parameters[13]. With PMA stimulation, PMNLs in intestinal Behçet's disease and in active ulcerative colitis showed significantly higher CL activities than control cells. Neutrophils in Crohn's disease and in inactive ulcerative colitis were also activated but not statistically significantly. On the other hand, monocytes were significantly activated by PMA stimulation in Crohn's disease and in active ulcerative colitis, but not in Behçet's disease and in inactive ulcerative colitis[124]. Whole blood and isolated monocytes from patients with Crohn's disease were also found to be activated by opsonized zymosan stimulation, whereas the CL activity of isolated neutrophils did not differ from that of controls[71, 96]. The high CL activity of patient whole blood conflicts with the result obtained from isolated neutrophils. It has indeed been shown recently that the procedure used for the separation of PMNLs has an important influence on the interpretation of results from in vitro studies of these cells[66]. Neutrophil CL stimulated with opsonized zymosan was found to be significantly higher in patients than in normal controls. There were no significant differences between the patients with ulcerative colitis and those with Crohn's disease. Neutrophil CL did not correlate with either therapy or disease location. These results were obtained using a two-step procedure for PMNL separation. With one-step separation the CL activity of PMNLs was depressed in patients. It was demonstrated that this disparity was caused by the elimination of low-density neutrophils with high CL production by the one-step procedure[66].

It is possible that serum contains a substance which stimulates neutrophil CL, but this was not clearly demonstrated by experiments where control cells were incubated with patient or control sera. No difference between sera from patients and healthy persons was observed[48, 66]. We have, however, found that sera from children with symptoms of gastrointestinal disorders, activated control cells when incubated with gliadin or milk proteins, and the peak CL emission correlated well with the antigliadin or antimilk protein IgA and IgG content of the sera. Moreover, when the antigens were added directly to whole blood samples from patients, the whole blood CL correlated with the specific IgG content but not with the specific IgA content of the plasma[81]. This led us to conclude that in patients a subpopulation of PMNLs possessing IgA receptors has left the circulation and is located in the intestine.

In summary, PMNLs and possibly monocytes are systemically activated in inflammatory bowel disease patients. The activation is not dependent on the type of disease, its location or activity. Patient sera do not contain large activating immune complexes, but exposure to food antigens, together with specific antibodies, may activate the cells in the intestine.

Neoplasia

Monocytes but not granulocytes showed enhanced CL activity in cancer patients with solid tumours. It was also evident that monocytes from patients with disseminated disease behaved differently from cells from those with nonmetastatic disease[22, 23, 130]. On the other hand, in leukaemia patients both monocytes and neutrophils were affected[27, 35, 47, 128]. We have successfully used the CL emission measurement from whole blood to monitor the graft take after allogeneic bone marrow transplantation in leukaemia patients[74, 104, 125].

Reproductive immunology

Whole blood CL activity varied regularly during the menstrual cycle[64]. We have observed four consecutive reproducible peaks of CL, the first three when the estradiol, then the LH and then the progesterone levels started to rise, and the fourth one when the rise in progesterone concentration leveled off. These values could be informative, especially for women with difficulties in fertilization, and they could also be used for choosing the time of oocyte aspiration for in vitro fertilization. Similar changes in CL were also seen in leukocyte samples from cow's milk[82]. After fertilization the progesterone level in plasma remained high. CL activities of leukocytes from pregnant women were higher than those from controls[115, 117]. In endometriosis, the phagocytes from both peripheral blood and peritoneum showed increased CL activity which suggested that endometriosis may be a systemic rather than a local disorder[140].

Seminal phagocytes may play an important role in male fertility. CL measurements from semen provided a convenient method for routine diagnosis of leukocytospermia[75].

Comparative immunology

Mostly conventional laboratory test animals and domestic animals have been used for studies of phagocyte CL. This work has been mainly concerned with the pathophysiological state of the animal as a model system for human defence reactions. No studies have been published on the effects of basic factors that regulate the animal life in the wild, like winter sleep, hibernation, light rhythm, exposure to cold and heat, nutritional status, mating period etc.

Apart from mammals, only fishes have been extensively studied. Fish phagocyte CL has been investigated in relation to stress[12] and stress hormones[19, 51], drugs[62, 134], pollutants[135], opsonization[138], immunization[112, 138] and bacterial strains[123], among other topics. Phagocytes have been isolated from peripheral blood, pronephros (the primary lymphoid organ and haematopoietic tissue in fish), spleen and peritoneum, and CL in diluted whole blood has also been measured[137]. Most of the studies have been made with fishes in cultivation, e.g. rainbow trout. The results suggest that photon emission in fish leukocytes, its origin and regulation, and the effect of the host's pathophysiological state on it, are essentially the same as in mammalian cells. This leads to the conclusion that advanced defence mechanisms based on phagocytes evolved early in vertebrate evolution.

A few studies have been made with birds, namely with poultry[42, 59]. In molluscs, phagocyte CL emission is reported to exist in oysters[14, 50, 73] and in snails[5, 6, 39]. Two reports of amphibian CL have been published, to our knowledge[78, 103]. We have studied the acclimatisation of *Rana pipiens*[78]. In all the other phyla and classes, and in vast majority of animal species the phenomenon remains totally unexplored.

1 Abramson, J. S., Giebink, G. S., Mills, E. L., and Quie, P. G., Polymorphonuclear leukocyte dysfunction during influenza virus infection in chinchillas. J. infect. Dis. *143* (1981) 836–845.

2 Abramson, J. S., Mills, E. L., Sawyer, M. K., Regelman, W. R., Nelson, J. D., and Quie, P. G., Recurrent infections and delayed separation of the umbilicar cord in an infant with abnormal phagocytic cell locomotion and oxidative response during particle phagocytosis. J. Ped. *99* (1981) 887–894.

3 Abramson, J. S., Giebink, G. S., and Quie, P. G., Influenza, a virus-induced polymorphonuclear leukocyte dysfunction in the pathogenesis of experimental pneumococcal otitis media. Infect. Immun. *36* (1982) 289–296.

4 Abramson, J. S., Wagner, M. P., Ralston, E. P., Wei, Y., and Wheeler, J. G., The ability of polymorphonuclear leukocyte priming agents to overcome influenza, a virus-induced cell dysfunction. J. Leukocyte Biol. *50* (1991) 160–166.

5 Adema, C. M., van Deutekom-Mulder, E. C., van der Knaap, W. P. W., Meuleman, E. A., and Sminia, T., Generation of oxygen radicals in haemocytes of the snail *Lymnaea stagnalis* in relation to the rate of phagocytosis. Dev. comp. Immun. *15* (1991) 17–26.

6 Adema, C. M., Harris, R. A., and van Deutekom-Mulder, E. C., A comparative study of hemocytes from six different snails morphology and functional aspects. J. Invert. Path. *59* (1992) 24–32.

7 Allen, R. C., Stjernholm, R. L., Reed, M. A., Harper, T. B., Gupta, S., Steele, R. H., and Waring, W. W., Correlation of metabolic and chemiluminescent responses of granulocytes from three female siblings with chronic granulomatous disease. J. immun. Dis. *136* (1977) 510–518.

8 Allen, R. C., Mills, E. L., McNitt, T. R., and Quie, P. G., Role of myeloperoxidase and bacterial metabolism in chemiluminescence of granulocytes from patients with chronic granulomatous disease. J. infect. Dis. *144* (1981) 344–348.

9 Andersen, O. K., Revhaug, A., Lundgren, T. I., and Giercksky, K.-E., Metabolic burst and motility in granulocytes following trauma and endotoxemia in the anaesthetized pig. Acta chir. scand. *152* (1986) 641–645.

10 Anderson, R., Theron, A. J., and Ras, G. J., Ascorbic acid neutralizes reactive oxidants released by hyperactive phagocytes from cigarette smokers. Lung *166* (1988) 149–159.

11 Anderson, R., Theron, A. J., Richards, G. A., Myer, M. S., and van Rensburg, A. J., Passive smoking by humans sensitizes circulating neutrophils. Am. Rev. Respir. Dis. *144* (1991) 570–574.

12 Angelidis, P., Baudin-Laurencin, F., and Youinou, P., Stress in rainbow trout *Salmo gairdneri* effects upon phagocyte chemiluminescence circulating leukocytes and susceptibility to *Aeromonas salmonicida*. J. Fish Biol. *31* (suppl. A) (1987) 113–122.

13 Anton, P. A., Targan, S. R., and Shananan, F., Increased neutrophil receptors for and response to the proinflammatory bacterial peptide formyl-methionyl-leucyl-phenylalanine in Crohn's disease. Gastroenterology *97* (1989) 20–28.

14 Bachere, E., Hervio, D., and Mialhe, E., Luminol dependent chemiluminescence by hemocytes of two marine bivalves *Ostrea edulis* and *Crassostrea gigas*. Dis. aquat. Org. *11* (1991) 173–180.

15 Barañao, R. I., Garberi, J. C., Tesone, P. A., and Rumi, L. S., Evaluation of neutrophil activity and circulating immune complexes levels in diabetic patients. Horm. Metab. Res. *19* (1987) 371–374.

16 Barañao, R. I., Rumi, L. S., Tesone, P. A., and Foglia, V. G., Some characteristics of neutrophils from diabetic patients and their relation to the levels of circulating immune complexes. Acta diabet. lat. *25* (1988) 13–23.

17 Barbour, A. G., Allred, C. D., Solberg, C. O., and Hill, H. R., Chemiluminescence by polymorphonuclear leukocytes from patients with active bacterial infection. J. infect. Dis. *141* (1980) 14–26.

18 Bass, D. A., Olbrantz, P., Szedja, P., Seeds, M. C., and McCall, C. E., Subpopulations of neutrophils with increased oxidative product formation in blood of patients with infection. J. Immun. *136* (1986) 860–866.

19 Bayne, C. J., and Levy, S., Modulation of the oxidative burst in trout myeloid cells by adrenocorticotropic hormone and catecholamines: mechanism of action. J. Leukocyte Biol. *50* (1991) 554–560.

20 Bektas, S., Goetze, B., and Speer, C. P., Decreased adherence, chemotaxis and phagocytic activities of neutrophils from preterm neonates. Acta paediatr. scand. *79* (1990) 1031–1038.

21 Bondestam, M., Håkansson, L., Foucard, T., and Venge, P., Defects in polymorphonuclear neutrophil function and susceptibility to infection in children. Scand. J. clin. Lab. Invest. *46* (1986) 685–694.

22 Braun, D. P., Harris, J. E., Maximovich, S., Marder, R., and Lint, T. F., Chemiluminescence in peripheral blood mononuclear cells of solid tumor cancer patients. Cancer Immun. Immunother. *12* (1981) 31–37.

23 Braun, D. P., De Boer, K. P., and Harris, J. E., Chemiluminescence, suppression and cytotoxicity in peripheral blood mononuclear cells from solid tumor cancer patients. Cancer Immun. Immunother. *14* (1982) 86–91.

24 Busoni, P., Sarti, A., De Martino, M., Graziani, E., and Santoro, S., The effect of general and regional anesthesia on oxygen-dependent microbicidal mechanisms of polymorphonuclear leukocytes in children. Anesth. Analg. *67* (1988) 453–456.

25 Calhoun, W. J., Salisbury, S. M., Chosy, L. W., and Busse, W. W., Increased alveolar macrophage chemiluminescence and airspace cell superoxide production in active pulmonary sarcoidosis. J. Lab. clin. Med. *112* (1988) 147–156.

26 Calhoun, W. J., Enhanced reactive oxygen species metabolism of air space cells in hypersensitivity pneumonitis. J. Lab. clin. Med. *117* (1991) 443–452.

27 Candido, A., Rossi, P., Meo, F., Lippa, S., Littarru, G., and De Sole, P., A whole blood chemiluminescence study in some hematological patients. Haematologica *68* (1983) 478–486.

28 Cassidy, L. F., Lyles, D. S., and Abramson, J. S., Depression of polymorphonuclear leukocyte functions by purified influenza virus hemagglutinin and sialic acid-binding lectins. J. Immun. *142* (1989) 4401–4406.

29 Chusid, M. J., Shea, M. L., and Sarff, L. D., Determination of post-transfusion granulocyte kinetics by chemiluminescence in chronic granulomatous disease. J. Lab. clin. Med. *95* (1980) 168–174.

30 Chusid, M. J., Sohnle, P. G., Fink, J. N., and Shea, M. L., A genetic defect of granulocyte oxidative metabolism in a man with disseminated aspergillosis. J. Lab. clin. Med. *97* (1981) 730–738.
31 Cluzel, M., Damon, M., Chanez, P., Bousquet, J., De Paulet, A. C., Michel, F. B., and Godard, Ph., Enhanced alveolar cell luminol-dependent chemiluminescence in asthma. J. Allergy clin. Immun. *80* (1987) 195–201.
32 Coble, B.-I., Dahlgren, C., Molin, L., and Stendahl, O., Neutrophil function in psoriasis: effects of retinoids. Acta dermatol. vener. *67* (1987) 481–490.
33 Coble, B.-I., Brieheim, G., Dahlgren, C., and Molin, L., Function of exudate neutrophils from skin in psoriasis. Int. Archs Allergy appl. Immun. *85* (1988) 398–403.
34 Croft, K. D., Beilin, L. J., Vandongen, R., Rouse, I., and Masarei, J., Leukocyte and platelet function and eicosanoid production in subjects with hypercholesterolaemia. Atherosclerosis *83* (1990) 101–109.
35 Dammacco, F., Miglietta, A., and Perfetto, S. C., Impaired chemiluminescence response by neutrophils in patients with multiple myeloma. Scand. J. Haemat. *37* (1986) 289–295.
36 DeChatelet, L. R., and Shirley, P. S., Evaluation of chronic granulomatous disease by a chemiluminescence assay of microliter quantities of whole blood. Clin. Chem. *27* (1981) 1739–1741.
37 Descamps-Latscha, B., Nguyen, A. T., and Feutren, G., Phagocyte oxidative metabolism in cyclosporine- or placebo-treated patients with insulin-dependent (type I) diabetes mellitus of recent onset. J. Autoimmun. *3* (1990) 201–213.
38 Dezzutti, C. S., Lafrado, L. J., Lewis, M. G., and Olsen, R. G., Inhibition of phorbol ester-induced neutrophil chemiluminescence by FeLV. Arch. Virol. *111* (1990) 75–85.
39 Dikkeboom, R., van der Knaap, W. P. W., van den Bovenkamp, W., Tijnagel, J. M. G. H., and Bayne, C. J., The production of toxic oxygen metabolites by hemocytes of different snail species. Dev. comp. Immun. *12* (1988) 509–520.
40 Driscoll, M. S., Thomas, V. L., Ramamurthy, R. S., and Casto, D. T., Longitudinal evaluation of polymorphonuclear leukocyte chemiluminescence in premature infants. J. Pediatr. *116* (1990) 429–434.
41 Edwards, S. W., Hart, C. A., Davies, J. M., Pattison, J., Hughes, V., and Sills, J. A., Case report. Impaired neutrophil killing in a patient with defective degranulation of myeloperoxidase. J. clin. Lab. Immun. *25* (1988) 201–206.
42 Emergy, D. A., Nagaraja, K. V., Sivanandan, V., Lee, B. W., Zhang, C. L., and Newman, J. A., Endotoxin lipopolysaccharide from *Escherichia coli* and its effects on the phagocytic function of systemic and pulmonary macrophages in turkeys. Avian Dis. *35* (1991) 901–909.
43 Engels, W., Grauls, G., Lemmens, P. J., Mullers, W. J., and Bruggeman, C. A., Influence of a cytomegalovirus infection on functions and arachidonic acid metabolism of rat peritoneal macrophages. J. Leukocyte Biol. *45* (1989) 466–473.
44 van Epps, D. E., Goodwin, J. S., and Murphy, S., Age dependent variations in polymorphonuclear leukocyte chemiluminescence. Infect. Immun. *22* (1978) 57–61.
45 Ertürk, M., Jennings, R., Oxley, K. M., and Hastings, M. J. G., Effect of influenza a on phagocytic cell function. Med. Microbiol. Immun. *178* (1989) 199–209.
46 Eschenbach, C., Chemilumineszenz phagocytierender neutrophiler Granulocyten von Neugeborenen mit bakteriellen Infektionen. Monatsschr. Kinderheilkd. *129* (1981) 640–644.
47 Estevez, M., Ballart, I. J., De Macedo, M. P., Magnasco, H., Nicastro, M. A., and Sen, L., Dysfunction of monocytes in Hodgkin's disease by excessive production of PGE-2 in long-term remission patients. Cancer *62* (1988) 2128–2133.
48 Faden, H., and Rossi, T. M., Chemiluminescent response of neutrophils from patients with inflammatory bowel disease. Dig. Dis. Sci. *30* (1985) 139–142.
49 Fisher, M., Upchurch, K. S., Levine, P. H., Johnson, M. H., Vaudreuil, C. H., Natale, A., and Hoogasian, J. J., Effects of dietary fish oil supplementation on polymorphonuclear leukocyte inflammatory potential. Inflammation *10* (1986) 387–392.
50 Fisher, W. S., Wishkovsky, A., and Chu, F. E., Effects of tributyltin on defence-related activities of oyster hemocytes. Arch. envir. Contam. Toxic. *19* (1990) 354–360.
51 Flory, C. M., and Bayne, C. J., The influence of adrenergic and cholinergic agents on the chemiluminescent and mitogenic responses of leukocytes from the rainbow trout *Oncorhynchus mykiss*. Dev. comp. Immun. *15* (1991) 135–142.
52 Follin, P., Brieheim, G., Sandstedt, S., and Dahlgren, C., Human neutrophil chemiluminescence and f-meth-leu-phe receptor exposure in bacterial infections. APMIS *97* (1989) 585–590.
53 Frigieri, L., Di Mario, G., Fresu, R., Grilli, N., Pizzoli, C., Pagiari, G., and De Sole, P., Chemiluminescence of macrophages from bronchoalveolar lavage, in: Bioluminescence and Chemiluminescence. Current Status, pp. 281–284. Eds P. E. Stanley and L. J. Kricka. John Wiley & Sons, Chichester, England 1991.
54 Friis-Møller, A., and Kharazmi, A., Neutrophil response, serum opsonic activity, and precipitating antibodies in human infection with *Actinobacillus hominis*. APMIS *96* (1988) 1023–1028.
55 Gale, R., Bertouch, J. V., Bradley, J., and Roberts-Thompson, P. J., Direct activation of neutrophil chemiluminescence by rheumatoid sera and synovial fluid. Ann. rheum. Dis. *42* (1983) 158–162.
56 Graft, D. F., Mischler, E., Farrell, P. M., and Busse, W. W., Granulocyte chemiluminescence in adolescent patients with cystic fibrosis. Am. Rev. Respir. Dis. *125* (1982) 540–543.
57 Gyllenhammar, H., Ringertz, B., Becker, W., Svensson, J., and Palmblad, J., Essential fatty acid deficiency in rats: effects on arachidonate metabolism, generation of cyclooxygenase products and functional responses in neutrophils. Immunology Lett. *13* (1986) 185–189.
58 Gyllenhammar, H., Palmblad, J., Ringertz, B., Hafström, I., and Borgeat, P., Rat neutrophil function, and leukotriene generation in essential fatty acid deficiency. Lipids *23* (1988) 89–95.
59 Harmon, B. G., and Glisson, J. R., Disassociation of bactericidal and fungistatic activities from the oxidative burst of avian macrophages. Am. J. vet. Res. *51* (1990) 71–75.
60 Jacobs, R. F., Kiel, D. P., and Balk, R. A., Alveolar macrophage function in a canine model of endotoxin-induced lung injury. Am. Rev. Respir. Dis. *134* (1986) 745–751.
61 James, D. W., Betts, W. H., and Cleland, L. G., Chemiluminescence of polymorphonuclear leukocytes from rheumatoid joints. J. Rheumat. *10* (1983) 184–189.
62 Kajita, Y., Sakai, M., Atsuta, S., and Kobayashi, M., The immunomodulatory effects of levamisole on rainbow trout *Onchorhynchus mykiss*. Fish Path. *25* (1990) 93–98.
63 Kalra, J., Chaundry, A. K., and Prasad, K., Increased production of oxygen free radicals in cigarette smokers. Int. J. exp. Path. *72* (1991) 1–7.
64 Kankaanpää, A., and Lilius, E.-M., Leukocyte activity and the ovarian cycle. XXXIst International congress of physiological sciences, Helsinki, Finland, July 1991, p. 1442.
65 Kantar, A., Wilkins, G., Swoboda, B., Littarru, G. P., Bertoli, E., Catassi, C., Coppa, G., and Giorgi, L., Alterations of the respiratory burst of polymorphonuclear leukocytes from diabetic children. Acta paediatr. scand. *79* (1990) 535–541.
66 Kelleher, D., Feighery, C., and Weir, D. G., Chemiluminescence by polymorphonuclear leukocyte subpopulations in chronic inflammatory bowel disease. Digestion *45* (1990) 158–165.
67 Kelly, C. A., Ward, C., Stenton, S. C., Hendrick, D. J., and Walters, E. H., Assessment of pulmonary macrophage and neutrophil function in sequential bronchoalveolar lavage aspirates in sarcoidosis. Thorax *43* (1988) 787–791.
68 Kiemle-Kallee, J., Kreipe, H., Radzun, H. J., Parwaresch, M. R., Auerswald, U., Magnussen, H., and Barth, J., Alveolar macrophages in idiopathic pulmonary fibrosis display a more monocyte like immunophenotype and an increased release of free oxygen radicals. Eur. J. Respir. Dis. *4* (1991) 400–406.
69 Kitahara, M., Eyre, H. J., Lynch, R. E., Rallison, M. L., and Hill, H. R., Metabolic activity of diabetic monocytes. Diabetes *29* (1980) 251–256.
70 Kitahara, M., Eyre, H. J., Simonian, Y., Atkin, C. L., and Hasstedt, S. J., Hereditary myeloperoxidase deficiency. Blood *57* (1981) 888–893.
71 Kitahora, T., Suzuki, K., Asakura, H., Yoshida, T., Suematsu, M., Watanabe, M., Aiso, S., and Tsuchiya, M., Active oxygen species generated by monocytes and polymorphonuclear cells in Crohn's disease. Dig. Dis. Sci. *33* (1988) 951–955.
72 Kokot, K., Schaefer, R. M., Teschner, M., Gilge, U., Plass, R., and Heidland, A., Activation of leukocytes during physical exercise. Adv. exp. Med. Biol. *240* (1988) 57–63.
73 Larson, K. G., Roberson, B. S., and Hetrick, F. M., Effect of environmental pollutants on the chemiluminescence of hemocytes from the American oyster *Crassostrea virginica*. Dis. aquat. Org. *6* (1989) 131–136.

74 Leino, L., Lilius, E.-M., Nikoskelainen, J., Pelliniemi, T.-T., and Rajamäki, A., Neutrophils are responsible for the reappearance of chemiluminescence after allogeneic bone marrow transplantation. Bone Marrow Transplant. *6* (1990) 391–394.

75 Leino, L., and Virkkunen, P., An automated chemiluminescence test for diagnosis of leukocytospermia. Int. J. Androl. *14* (1991) 271–277.

76 Leirisalo-Repo, M., Lauhio, A., and Repo, H., Chemotaxis and chemiluminescence responses of synovial fluid polymorphonuclear leucocytes during acute reactive arthritis. Ann. rheum. Dis. *49* (1990) 615–619.

77 Lewis, M. G., Duska, G. O., Stiff, M. I., Lafrado, L. J., and Olsen, R. G., Polymorphonuclear leukocyte dysfunction associated with feline leukaemia virus infection. J. gen. Virol. *67* (1986) 2113–2118.

78 Lilius, E.-M., Leukocytes as immunosensors, in: Biological Luminescence, pp. 449–459. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, Singapore 1990.

79 Lilius, E.-M., Marnila, P., and Metsä-Ketelä, T., Effect of fish oil diet on the functions of rat leukocytes and heart. Xth Symposium of Food Chemistry, Triacyl Glycerols and Nutrition. University of Turku, Finland, 4./5.1.1990.

80 Lilius, E.-M., Mäki, A.-L., Proskin, J., and Rajamäki, A., Leukocytes as immunosensors: Whole blood chemiluminescence (CL) in human leukaemias, in: Bioluminescence and Chemiluminescence. New Perspectives, pp. 53–56. Eds J. Schölmerlich, R. Andreesen, A. Kapp, M. Ernst and W. G. Woods. John Wiley & Sons, Chichester, England 1987.

81 Lilius, E.-M., Nykänen, J., and Ståhlberg, M.-R., Leukocytes as immunosensors: a screening method for food intolerances, in: Bioluminescence and Chemiluminescence. New Perspectives, pp. 145–148. Eds J. Schölmerlich, R. Andreesen, A. Kapp, M. Ernst and W. G. Woods. John Wiley & Sons, Chichester, England 1987.

82 Lilius, E.-M., and Pesonen, U.-M., Use of inflammatory cell activities in bovine milk to diagnose mastitis. Am. J. vet. Res. *51* (1990) 1527–1533.

83 Lindena, J., Burkhardt, H., and Dwenger, A., Mechanisms of non-opsonized zymosan-induced and luminol-enhanced chemiluminescence in whole blood and isolated phagocytes. J. clin. Chem. clin. Biochem. *25* (1987) 765–778.

84 Magaro, M., Altomonte, L., Zoli, A., Mirone, L., De Sole, P., Di Mario, G., Lippa, S., and Oradei, A., Influence of diet with different lipid composition on neutrophil chemiluminescence and disease activity in patients with rheumatoid arthritis. Ann. rheum. Dis. *47* (1988) 793–796.

85 Majima, T., Minegishi, N., Nagatomi, R., Ohashi, Y., Tsuchiya, S., Kobayashi, K., and Konno, T., Unusual expression of IgG Fc receptors on peripheral granulocytes from patients with leukocyte adhesion deficiency (CD11/CD18). J. Immun. *145* (1990) 1694–1699.

86 Marnila, P. J., Lagerspetz, K. Y. H., and Lilius, E.-M., Leukocyte activity and thermoregulation. XXXIst International congress of physiological sciences, Helsinki, Finland, July 1991, p. 3576.

87 Martin, R. R., Lawrence, E. C., Teague, R. B., Gottlieb, M. S., and Putman, M., Chemiluminescence of lung macrophages and blood leukocytes in sarcoidosis. Am. Rev. Respir. Dis. *133* (1986) 298–301.

88 McCarthy, J. P., Bodroghy, R. S., Jahrling, P. B., and Sobocinski, P. Z., Differential alterations in host peripheral polymorphonuclear leukocyte chemiluminescence during the course of bacterial and viral infections. Infect. Immun. *30* (1980) 824–831.

89 Mealy, K., O'Farrelly, C., Stephens, R., and Freighery, C., Impaired neutrophil function during anesthesia and surgery is due to serum factors. J. Surg. Res. *43* (1987) 393–397.

90 Michel, F., Rudent, A., Labarre, C., Quero, A. M., Zalisz, R., and Smets, P., Effects of RU 41740 aerosol treatment on mouse bronchoalveolar cells, and protection afforded against influenza virus infection. Int. J. Immunopharmac. *9* (1987) 775–781.

91 Mills, E. L., Thompson, T., Björksten, B., Filipovich, D., and Quie, P. G., The chemiluminescence response and bactericidal activity of polymorphonuclear neutrophils from newborns and their mothers. Pediatrics *63* (1979) 429–434.

92 Mills, E. L., Rholl, K. S., and Quie, P. G., Luminol-amplified chemiluminescence: a sensitive method for detecting the carrier state in chronic granulomatous disease. J. clin. Microbiol. *12* (1980) 52–56.

93 Miyata, H., Moriguchi, N., and Kinoshita, T., The chemiluminescence response of polymorphonuclear leukocytes from febrile patients. Clin. chim. Acta *173* (1988) 337–342.

94 Nutter, R. L., Kettering, J. D., Aprecio, R. M., Weeks, D. A., and Gridley, D. S., Effects of dietary fat and protein on DMH-induced tumor development and immune responses. Nutr. Cancer *13* (1990) 141–152.

95 O'Neill, B., and Leonard, B. E., Abnormal zymosan-induced neutrophil chemiluminescence as a marker of depression. J. affect. Disord. *19* (1990) 265–272.

96 Okabe, N., Kuroiwa, A., Fujita, K., Shibuya, T., Yao, T., and Okomura, M., Immunological studies on Crohn's disease VI. Increased chemiluminescent response of peripheral blood monocytes. J. clin. Lab. Immun. *21* (1986) 11–15.

97 Pauksens, K., Sjöling, J., and Venge, P., Chemiluminescence of polymorphonuclear leukocytes and whole blood during acute bacterial infection. Scand. J. infect. Dis. *21* (1989) 277–284.

98 Peden, D. B., Van Dyke, K., Ardekani, A., Mullett, M. D., Myerberg, D. Z., and Van Dyke, C., Diminished chemiluminescent responses of polymorphonuclear leukocytes in severely and moderately preterm neonates. J. Pediatr. *6* (1987) 904–906.

99 Pedersen, B. K., Tvede, N., Klarlund, K., Christensen, L. D., Hansen, F. R., Galbo, H., Kharazmi, A., and Halkjär-Kristensen, J., Indomethacin in vitro and in vivo abolishes post-exercise suppression of natural killer cell activity in peripheral blood. Int. J. Sports Med. *11* (1990) 127–131.

100 Perttilä, J., Lehtonen, O.-P., Salo, M., and Tertti, R., Effects of coronary bypass surgery under high-dose fentanyl anaesthesia on granulocyte chemiluminescence. Br. J. Anaesth. *58* (1986) 1027–1030.

101 Perttilä, J., Lilius, E.-M., and Salo, M., Effects of anaesthesia and surgery on serum opsonic capacity. Acta anaesth. scand. *30* (1986) 173–176.

102 Perttilä, J., Salo, M., Peltola, O., and Irjala, K., Changes in granulocyte chemiluminescence and plasma fibronectin concentrations following major blunt trauma. Eur. Surg. Res. *20* (1988) 211–219.

103 Plytycz, B., and Bayne, C. J., PMA-induced chemiluminescence of amphibian leukocytes. Dev. comp. Immun. *11* (1987) 245–250.

104 Rajamäki, A., Lilius, E.-M., Nikoskelainen, J., Proskin, J., Salmi, T. T., and Toivanen, A., Luminol enhanced chemiluminescence of peripheral blood leukocytes as an early indicator of graft take after allogeneic bone marrow transplantation in patients with acute myelogenous leukemia. Med. Oncol. Tumor Pharmacother. *5* (1988) 99–102.

105 Regelmann, W. E., Lunde, N. M., Porter, P. T., and Quie, P. G., Increased monocyte chemiluminescence in cystic fibrosis patients and in their parents. Ped. Res. *20* (1986) 619–622.

106 Richards, G. A., Theron, A. J., van der Merwe, C. A., and Anderson, R., Spirometric abnormalities in young smokers correlate with increased chemiluminescence responses of activated blood phagocytes. Am. Rev. Respir. Dis. *139* (1989) 181–187.

107 Richards, G. A., Theron, A. J., van Rensburg, C. E. J., van Rensburg, A. J., van der Merwe, C. A., Kuyl, J. M., and Anderson, R., Investigation of the effects of oral administration of vitamin E and beta-carotene on the chemiluminescence responses and the frequency of sister chromatid exchanges in circulating leukocytes from cigarette smokers. Am. Rev. Respir. Dis. *142* (1990) 648–654.

108 Roberts, R. L., and Stiehm, E. R., Increased phagocytic cell chemiluminescence in patients with cystic fibrosis. Am. J. Dis. Child. *143* (1989) 944–950.

109 Rosen, H., and Klebanoff, S. J., Chemiluminescence and superoxide production by myeloperoxidase-deficient leukocytes. J. clin. Invest. *58* (1976) 50–60.

110 Rudent, A., Michel, F., Labarre, C., Quero, A. M., Zalisz, R., and Smets, P., Enhancement of broncoalveolar cell recovery and stimulation of alveolar macrophage chemiluminescence and resistance to influenza virus after treatment with RU 41821 aerosol. Antimicrob. Agents Chemother. *31* (1987) 920–924.

111 Røkke, O., Revhaug, A., and Giercksky, K.-E., Polymorphonuclear leukocyte activation after trauma: evidence for increased generation of oxygen free radicals in responce to in vitro endotoxin stimulation. Acta chir. scand. *155* (1989) 233–239.

112 Sakai, M., Kamiya, H., Atsuta, S., and Kobayashi, M., Immunomodulatory effects on rainbow trout *Oncorhynchus mykiss* injected with the extract of *Abalone haliotis* discus *hannai*. J. appl. Ichtyol. *7* (1991) 54–59.

113 Salo, M., Perttilä, J., and Lehtonen, O.-P., Granulocyte chemiluminescence in patients with postoperative infections. Arch. Surg. *123* (1988) 17–22.

114 Schopf, R. E., and Straussfeld, E., Stimulus-dependent increased generation of oxygen intermediates in monocytes and polymorphonuclear leukocytes in psoriasis. J. Invest. Dermat. *84* (1985) 73–76.

115 Selvaraj, R. J., Sbarra, A. J., Thomas, G. B., Cetrulo, C. L., and Mitchell, G. W., A microtechnique for studying chemiluminescence response of phagocytes using whole blood and its application to the evaluation of phagocytes in pregnancy. J. Reticuloendot. Soc. *31* (1982) 3–16.

116 Sheng, C. Y., and Tung, Y. L., Neutrophil chemiluminescence in burned patients. J. Trauma *27* (1987) 587–595.

117 Shibuya, T., Izuchi, K., Kuroiwa, A., Okabe, N., and Shirakawa, K., Study on nonspecific immunity in pregnant women: increased chemiluminescence response of peripheral blood phagocytes. Am. J. Reprod. Immun. Microbiol. *15* (1987) 19–23.

118 Shigeoka, A. O., and Hill, H. R., Case report. Recurrent pseudomonas infection associated with neutrophil dysfunction. Scand. J. infect. Dis. *10* (1978) 307–311.

119 Shigeoka, A. O., Charette, R. P., Wyman, M. L., and Hill, H. R., Defective oxidative metabolic responses of neutrophils from stressed neonates. J. Pediatr. *3* (1981) 392–398.

120 de Simone, C., de Sole, P., di Mario, G., Venier, A., Cerimele, D., and Serri, F., Reactive oxygen species production in circulating polymorphonuclear leukocytes in psoriasis. Acta dermat. vener. *146* (1989) 50–52.

121 Smith, J. A., Telford, R. D., Mason, I. B., and Weideman, M. J., Exercise, training and neutrophil microbicidal activity. Int. J. Sports Med. *11* (1990) 179–187.

122 Solberg, C. O., Kalager, T., Hill, H. R., and Glette, J., Polymorphonuclear leukocyte function in bacterial and viral infections. Scand. J. infect. Dis. *14* (1982) 11–18.

123 Stave, J. W., Cook, T. M., and Robertson, B. S., Chemiluminescent responses of striped bass *Morone saxatilis* walbaum phagocytes to strains of *Yersinia ryckeri*. J. Fish Dis. *10* (1987) 1–10.

124 Suematsu, M., Suzuki, M., Kitahora, T., Miura, S., Suzuki, K., Hibi, T., Watanabe, M., Nagata, H., Asakura, H., and Tsuchiya, M., Increased respiratory burst of leukocytes in inflammatory bowel disease – the analysis of free radical generation by using chemiluminescence probe. J. clin. Lab. Immun. *24* (1987) 125–128.

125 Toivanen, A., Nikoskelainen, J., Lilius, E.-M., Salmi, T. T., Kätkä, K., Pelliniemi, T.-T., Mäki, A.-L., and Rajamäki, A., Peripheral blood chemiluminescence as an early indicator of successful bone marrow transplantation. Transplant. Proc. *19* (1987) 2745–2746.

126 Tono-oka, T., Matsumoto, T., Ueno, N., Yashiki, N., and Matsumoto, S., Chemiluminescence of whole blood. II. Application to clinical examination of phagocytic functions of whole blood from various types of disease. Clin. Immun. Immunopath. *29* (1983) 333–340.

127 Trulson, A., Nilsson, S., and Venge, P., Lucigenin-enhanced chemiluminescence in blood is increased in cancer. Am. J. clin. Path. *91* (1989) 441–445.

128 Tullgren, O., Giscombe, R., Holm, G., Johansson, B., Ellstedt, H., and Björkholm, M., Increased luminol-enhanced chemiluminescence of blood monocytes and granulocytes in Hodgkin's disease. Clin. exp. Immun. *85* (1991) 436–440.

129 Usmani, S. S., Schlessel, J. S., Sia, C. G., Kamran, S., and Orner, S. D., Polymorphonuclear leukocyte function in the preterm neonate: effect of chronologic age. Pediatrics *87* (1991) 675–679.

130 Wallaert, B., Ramon, P., Fournier, E. C., Prin, L., Tonnel, A. B., and Voisin, C., Activated alveolar macrophage and lymphocyte alveolitis in extrathoracic sarcoidosis without radiological mediastinopulmonary involvement. Ann. N.Y. Acad. Sci. *465* (1986) 201–210.

131 Wallaert, B., Bonniere, B., Prin, L., Cortot, A., Tonnel, A. B., and Voisin, C., Primary biliary cirrhosis. Subclinical inflammatory alveolitis in patients with normal chest roentgenograms. Chest *90* (1986) 842–848.

132 Weening, R. S., Corbeel, L., de Boer, M., Lutter, R., van Zwieten, R., Hamers, M. N., and Roos, D., Cytochrome b deficiency in an autosomal form of chronic granulomatous disease. J. clin. Invest. *75* (1985) 915–920.

133 Winocour, P. H., Lenton, J., Puxty, J. A. H., and Anderson, D. C., Leukocyte microbicidal activity assessed by chemiluminescence in elderly non-insulin dependent diabetes mellitus. Diabetes Res. *9* (1988) 73–75.

134 Wishkovsky, A., Robertson, B. S., and Hetrick, F. M., In-vitro suppression of the phagocytic response of fish macrophages by tetracyclines. J. Fish Biol. *31* (suppl. A) (1987) 61–66.

135 Wishkovsky, A., Mathews, E. S., and Weeks, B. A., Effect of tributyltin on the chemiluminescent response of phagocytes from three species of estuarine fish. Arch. envir. Contam. Toxic. *18* (1989) 829–831.

136 Wong, C. W., Thompson, H. L., Thong, Y. H., and Thornton, J. R., Effects of strenous exercise stress on chemiluminescence response of equine alveolar macrophages. Equine vet. J. *22* (1990) 33–35.

137 Yamada, F., Kodama, H., Mikami, T., and Izawa, H., Chemiluminescence of rainbow trout *Salmo gairdneri* phagocytes and factors affecting their response. Jap. J. vet. Sci. *50* (1988) 1092–1098.

138 Yoshida, T., and Kitao, T., The opsonic effect of specific immune serum on the phagocytic and chemiluminescent response in rainbow trout *Onchorhynchus mykiss* phagocytes. Fish Path. *26* (1991) 29–34.

139 Zeidler, R. B., and Kim, H. D., Phagocytosis, chemiluminescence, and cell volume of alveolar macrophages from neonatal and adult pigs. J. Leukocyte Biol. *37* (1985) 29–43.

140 Zeller, J. M., Henig, I., Radwanska, E., and Dmowski, W. P., Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. Am. J. Reprod. Immun. Microbiol. *13* (1987) 78–82.

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