The Respiratory Burst and Lymphocyte Function

ARTHUR L. SAGONE, Jr.

1. INTRODUCTION

Recent studies have established that phagocytic cells generate several reactive oxygen species (ROS).[1–24] Normally, under resting or baseline conditions, unstimulated granulocytes and monocytes appear to release only minimal, and probably nontoxic, amounts of ROS. However, during an immunological response, phagocytic cells may have a burst in oxidative metabolism (a respiratory burst), resulting in an enhanced release of ROS at an inflammatory site.[25–27] Since lymphocytes are an essential part of the inflammatory response, the potent compounds released by phagocytic cells might modify the functional capacity of these cells. This chapter discusses the possible effects of the ROS produced by phagocytic cells during the respiratory burst on the functional capacity of human lymphocytes at a site of inflammation.

Two potential effects might be anticipated. First, the overall effect might result in enhanced lymphocyte activity. Under these circumstances, the stimulated phagocytic cell would provide a helper cell function. Alternatively, the compounds released could impair lymphocytes function. Under these conditions, the stimulated phagocytic cell would exhibit suppressor cell activity.

The potential interaction between the ROS released by phagocytic cells and lymphocytes at an inflammatory site appears to be markedly complex and the effects of ROS on the functional capacity of lymphocytes could be modified by a number of factors generated during an immune response. Under most circumstances, the stimulus that induces a metabolic burst in phagocytic cells also stimulates simultaneously other independent cellular events. These include chemotax-

ARTHUR L. SAGONE, Jr. • Department of Medicine and Pharmacology, Division of Hematology and Oncology, Ohio State University, College of Medicine, Columbus, Ohio 43210.

A. J. Sbarra et al. (eds.), *The Respiratory Burst and Its Physiological Significance*

is,[28,29] the release of lymphokines[30,31] and enzymes with diverse activity,[32,33] and the generation of prostaglandins and leukotrienes from arachidonic acid.[34–37]

In these complex systems, altered lymphocyte function could occur as a consequence of direct interaction of the ROS with the lymphocyte or indirectly due to the interaction of the ROS with another compound produced simultaneously by the phagocytic cell. For example, the ROS might react with a lymphokine, enzyme, or a prostaglandin, which then interacts with the lymphocyte resulting in an altered function. Furthermore, in some cases, the ROS could react with factors released from lymphocytes and required for optimal lymphocyte function.

Of particular importance in considering the effects of the ROS produced by phagocytic cells on the function of lymphoid cells is the relative sensitivity of subpopulations of these cells to oxidant damage.

2. CHARACTERISTICS OF THE METABOLIC BURST

2.1. Nature of the Metabolic Burst in Phagocytic Cells

We have discussed in detail the nature of the biochemical reactions associated with the metabolic burst occurring in the granulocyte following its activation by an appropriate stimulus (see Chapter 12, this volume). The metabolic burst is associated with an immediate release of several reactive oxygen species by these cells, including superoxide (O_2^-),[4] hydrogen peroxide (H_2O_2),[18] hydroxyl free radical (OH·),[38–40,42] hypochlorous acid,[19–21] and the hypochlorous-derived stable oxidants-chloramines.[21–23] The stimulation of monocytes is associated with a similar rapid activation of oxidative metabolism.[15,41] The effect of the tumor promoter phorbol myristate acetate (PMA) on the HMPS activity and H_2O_2 production of blood mononuclear cell suspensions containing approximately 20% monocytes is given in Fig. 1. As seen, there is prompt activation of the HMPS pathway, as indicated by a marked increase in [1-^{14}C]glucose oxidation, and H_2O_2 production as indicated by enhanced [^{14}C]formate oxidation.

Overall, most studies indicate that stimulated monocytes release lower amounts of ROS than granulocytes.[6,15,42–48] These include the production of H_2O_2, HOCl, and OH·.

In addition to granulocytes and monocytes, lymphocytes may also be exposed to macrophages at an inflammatory site, particularly in chronic infections. In this regard, there appear to be important differences in the oxidative metabolism of the macrophages compared to the other phagocytic cells. The functional and biochemical characteristics of animal macrophage from a variety of tissues has been the subject of considerable investigation.[49–54] These studies indicate an importance of O_2^-, H_2O_2, and possibly OH· radical in the functional capacity of these cells. However, macrophages appear to lack the capacity to produce HOCl even after activation because they have relatively little myeloperoxidase activity.[44,55] In contrast to animal macrophages, the oxidative metabolism of human macrophages is

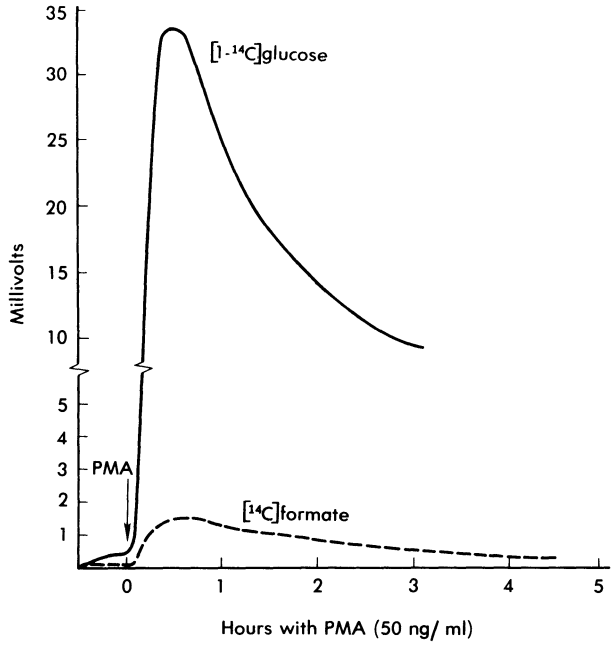

FIGURE 1. Oxidation of [1-^{14}C]glucose (——) and [^{14}C]formate (- - -) by suspensions of mononuclear cells (20 × 10^6 in 4.5 ml buffer). The curves represent a continuous measurement of $^{14}CO_2$ production from each substrate, using an ionization chamber electrometer system. The *y* axis indicates the electrical signal (in mV) generated by the $^{14}CO_2$ passing through the chamber. After steady state conditions were established, PMA was added as indicated by the arrow in a final concentration of 0.05 μg/ml. (From Sagone et al.[41])

less well delineated. Our knowledge of the biochemistry of this cell is derived primarily from the study of human alveolar macrophages.[42,44,56] Of importance is that these cells appear to release substantial amounts of O_2^- and H_2O_2, even under resting conditions. The alveolar macrophages derived by bronchopulmonary lavage from a normal person produces significant amounts of H_2O_2.[44,56] Similar to other phagocytic cells, the stimulation of human macrophages is associated with a burst in hexose monophosphate shunt pathway activity, increased oxygen consumption, and enhanced O_2^- and H_2O_2 production. Human macrophages appear to produce significantly less OH· than do other human phagocytic cells[42,44] and little HOCl. However, the O_2^- and H_2O_2 released by unstimulated macrophages might directly alter lymphocyte function while a similar alteration by PMN or monocytes may require stimulation of these cells.

Of particular interest is the capacity of phagocytic cells to become activated in vivo.[57–68] This activation is associated with an enhanced capacity to generate ROS. Phagocytic cells may become activated by a variety of mechanisms, such as exposure to interferon, lipopolysaccharide, and immune complexes.[69–74] While it may be somewhat confusing, the difference between activation and stimulation for

the purposes of this discussion should be noted. Stimulation indicates the enhanced immediate release of ROS, enzymes, and so on, following the interaction of a phagocytic cell with an appropriate stimulus. Within this context, an activated cell, when stimulated, would release increased amounts of ROS, enzymes, lymphokines, and possibly new compounds compared with the nonactivated cells. Under some circumstances, however, the differences between stimulation and activation become somewhat hazy or arbitrary. For example, the resident human alveolar macrophage in healthy persons may already be activated as a consequence of chronic exposure to the noxious agents in our atmosphere.

2.2. Generation of ROS by Lymphocytes

Thus far, I have discussed the metabolic burst occurring in phagocytic cells following interaction with an appropriate stimulus. However, a fundamental physiological question arises: Does the lymphocyte or a specific subpopulation, such as T cells, B cells, or NK cells have the capacity to generate ROS in a manner similar to that of PMN, monocytes, and macrophages? Several groups have studied the oxidative metabolism of purified lymphocyte preparations.[15,45,46,48] Agents that stimulate the oxidative metabolism of phagocytic cells do not stimulate the oxidative metabolism of lymphocytes. This includes agents such as PMA and merezein, which do not require specific membrane receptors.[48] Therefore, most evidence indicates that human lymphocytes lack NADPH oxidase activity and do not have the capacity to undergo an immediate burst in oxidative metabolism with the generation and a release of ROS characteristic of phagocytic cells.

Whereas lymphocytes do not appear to have the immediate burst in oxidative metabolism following interaction with a variety of stimuli the stimulation of oxidative metabolism does occur in lymphocytes under some circumstances. For example, it has been known for some time that lymphocyte cultures undergoing lymphoblastic transformation to nonspecific mitogens have a marked augmentation of HMPS activity, which appears to start late the first day of culture and is greatest during the second day.[75–78] However, it is not clear whether the enhanced HMPS response is associated with the increased cellular production of ROS or relates to a specific requirement of the HMPS for cellular division.

There is some evidence that lymphocytes may produce OH· or another ROS under some conditions.[79–81] The addition of hydroxyl scavengers to mitogen-stimulated lymphocyte cultures impairs their proliferation, suggesting the generation of OH· under these conditions.[79] It has been suggested that OH· may be related to the NK activity of lymphocytes.[80] This conclusion is based on the capacity of hydroxyl scavengers to inhibit the NK activity of lymphocyte cultures. As discussed in Chapter 12 (this volume), OH· might be produced in lymphocytes during arachidonic metabolism or by their microsomal enzyme systems. Thus far, lymphocytes have not been shown to produce OH· directly using specific assays that measure the production of OH· in biological systems.[80] This may relate to the sensitivity of the assays available to measure this ROS.

2.3. Stimuli That Induce a Metabolic Burst in Phagocytic Cells

A number of diverse stimuli are known to induce a respiratory burst in phagocytic cells in vitro. In this regard, specific membrane receptors are of particular importance in the stimulation of the oxidative metabolism of phagocytic cells. The most notable of these are the Fc and complement receptors present on the membrane of all phagocytic cells.[82–90] Stimulation of these receptors would appear to represent an important final common pathway for the stimulation of the oxidative metabolism of phagocytic cells in vitro by a variety of stimuli. These include soluble immune complexes, insoluble immune complexes,[3,32,33] opzonized infectious organism such as bacteria[5,90] and viruses,[91,92] and antibody-sensitized target cells such as RBC and tumor cells.[93–99] A similar activation of phagocytic cells may occur due to immune-complex absorption on lymphocytes.[100] These stimuli trigger a metabolic burst by stimulation of the Fc receptor. In most cases, the simultaneous absorption of complement components with the antigen–antibody complex appears to enhance the overall response apparently by simultaneous stimulation of the complement and Fc receptors.[82,90,94] However, activated complement alone may trigger a metabolic burst in phagocytic cells in vivo. The complement stimulation of granulocyte metabolism may be related to the pulmonary distress syndrome reported in patients following hemodialysis and WBC cell transfusions.[101] Furthermore, the activation of serum by snake venom is known to induce ARDS in animals.[102,103] In this regard, a well-studied model for the stimulation of a respiratory burst in phagocytic cells by complement is the incubation of these cells with serum opsonized zymosan particles.[15]

The respiratory burst can be also induced by other mechanisms. The chemotactic peptide FMLP appears to stimulate a metabolic burst and enzyme release in phagocytes by interaction with a specific membrane receptor,[29] while compounds such as PMA and merezein appear to stimulate oxidative metabolism by direct acfivation of the enzyme C-kinase, thereby bypassing the need for the membrane stimulation.[104]

It is reasonable to conclude that a number of these stimuli will induce release of ROS by phagocytes at an inflammatory site in vivo. Such reactions have been postulated in patients with autoimmune disorders, bacteria and viral infections, as well, as patients with neoplasia.

A few additional comments seem appropriate when considering the potential role of the ROS released by specific phagocytes in modifying lymphocytic function in vivo. The first question is whether there is selective activation of specific phagocytic cells by immune complexes or sensitized target cells in vivo. For example, monocytes appear to be able to interact with target cells sensitized by low concentrations of antibody.[82] Monocytes bind and lyse RBC sensitized by the serum antibodies present in patients with autoimmune hemolytic anemia (IGG subtypes 1 and 3),[105] and RBC sensitized by Rhogam antibody (ADCC).[99] The interaction is associated with a metabolic burst in the monocyte, suggesting that ADCC is mediated in part by the ROS generated by these cells.[99] By contrast, granulocytes do not

mediate ADCC in this system and are not stimulated. However, when granulocytes are incubated with target cells sensitized with high concentrations of antibody (i.e., human RBC sensitized with anti-A or anti-B antibody or rabbit RBC sensitized with sheep antirabbit antibodies), they mediate ADCC and have a metabolic burst similar to monocytes.[95,106]

Second, the capacity of unopsonized target cells to trigger an oxidative burst in phagocytic cells may vary considerably. The phagocytosis of unopsonized bacteria may be associated with some stimulation of the oxidative metabolism of the phagocyte cell although the degree response is much lower than that induced by opsonized organisms.[57,107] However, this is not always the case.[90,108] For example, histoplasma capsulation fails to trigger a metabolic burst in macrophages.[108] The failure of phagocytic cells to generate ROS may relate to the resistance of this organism to intracellular killing. Currently, there is little information concerning the capacity of viruses to induce the release of ROS in phagocytes. Abramson et al.[109] demonstrated that influenza virus stimulates a metabolic burst in both granulocytes and monocytes. We have demonstrated that the unopsonized mumps particles used for the mumps skin testing induce a oxidative metabolic burst in human monocyte suspensions but not granulocyte suspensions.[92] By contrast, we were unable to demonstrate a similar stimulation with inactivated feline leukemia virus[110] or inactivated poliovirus (unpublished observations). These observations suggest considerable variability in the capacity of some infectious agents and antigens to stimulate a oxidative burst in phagocytic cells. This seems to be a significant point, since the release of ROS by the phagocytic cell in addition to lymphokines might alter the primary immune response as a consequence of the capacity of the ROS to modify the functional capacity of lymphocytes.

3. EVIDENCE INDICATING THAT THE ROS PRODUCED BY PHAGOCYTIC CELLS MAY ALTER THE FUNCTIONAL CAPACITY OF HUMAN LYMPHOCYTES

Several in vitro model systems have addressed the effects of oxidant stress on the functional capacity of human lymphocytes. These systems include the effects of (1) periodate on lymphocyte proliferation, (2) hyperoxia on the proliferation of lymphocyte cultures, (3) the reactive oxygen species generated by enzyme systems on the T- and B-cell function of lymphocytes and finally, (4) oxidant injury in the radiation damage to lymphocytes. These systems have provided useful observations concerning the possible effects of the reactive oxygen species produced by phagocytic cells on a variety of lymphocyte function in vivo. For the most part, the results of these studies support the conclusion that oxidant stress to the lymphocyte in vivo may be associated with impaired T- and B-cell functions and further that these cells may be particularly sensitive to oxidant damage. The information generated in these model systems is discussed briefly in the following sections.

3.1. Effect of Hyperoxia on the Proliferation of Human Lymphocyte Cultures

Human blood lymphocytes that normally will not grow in culture can be induced to proliferate after stimulation with nonspecific mitogens such as phytohemogglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen. The proliferation of such cultures appears optimal under experimental conditions in which the oxygen tensions in the atmosphere used to grow the cultures approximate those occurring in vivo.[111–116] In 1968, Anderson demonstrated that PHA stimulated human lymphocyte cultures incubated under an atmosphere containing only a small increase in oxygen content (30%, rather than the usual 20%) had significantly impaired lymphoblastic transformation, and the cultures incubated under 100% oxygen had virtually no proliferation at all.[111,112] This impaired proliferation could not be explained by a decreased viability of the cells, and no obvious morphological abnormality could be demonstrated in the lymphocytes, by electron microscopic examination. Studies in our laboratory have similarly demonstrated that hyperoxia impairs the LBT of human lymphocytes.[115] We have also characterized the carbohydrate metabolism of lymphocytes cultures under these conditions. Under hyperoxia, unstimulated cultures have an increased rate of glycolysis, a slight decrease in [1-^{14}C]glucose oxidation, and a significant decrease in [6-^{14}C]glucose oxidation (Table I). When the rate of [1-^{14}C]glucose oxidation is corrected for the amount oxidized by Krebs, or tricarboxylic acid (TCA) cycle activity, there is no significant

TABLE I. Mean Rates of Glucose Consumptions and $^{14}CO_2$ Production of 3-day Unstimulated and Mitogen-Stimulated Lymphocyte Cultures under Air and Oxygen[a]

	Unstimulated			PHA-stimulated		
	Glucose consumption	[1-^{14}C]glucose	[6-^{14}C]glucose	Glucose consumption	[1-^{14}C]glucose	[6-^{14}C]glucose
			Cultures incubated under air, 5% CO_2			
Mean	83	5.7	0.90	644	17.0	1.5
SD	35	0.28	0.15	70	3.3	0.17
N	6	3	3	7	4	3
Range	49–101	5.5–6.0	0.7–1.0	552–746	14.4–21.8	1.4–1.7
			Cultures incubated under 95% oxygen, CO_2			
Mean	136	5.0	0.33	417	7.9	0.33
SD	51	0.25	0.06	55	1.4	0.06
N	6	3	3	7	4	3
Range	105–225	4.7–5.2	0.3–0.4	350–503	6.3–9.7	0.3–0.4
p^b	0.1	0.05	0.01	0.01	0.01	0.01

[a]Values are given in nanomoles/10^7 cell per hr.

[b]The data were analyzed according to tests for dependent or independent samples. Results are expressed as mean ± standard deviation (SD). (From Sagone.[115])

difference between the HMPS pathway activity of hyperoxic cultures and those grown under air. These data suggest that hyperoxia may cause subtle damage to the Krebs cycle of these cells without altering glycolysis or the activity of the HMPS pathway. Mitogen-stimulated cultures incubated under hyperoxia failed to demonstrate the augmentation in carbohydrate metabolism characteristic of cultures incubated under normal oxygen tensions.[75–77] This observation indicates that the impaired DNA synthesis of the cultures is not the only metabolic defect induced by hyperoxia and that the impaired carbohydrate metabolism of the hyperoxic cultures is an additional measure of the oxidant damage.

It is not clear whether the impaired carbohydrate metabolism induced by hyperoxia is the major cause for the impaired lymphocyte function. The effects of hyperoxia on the Krebs cycle activity of the unstimulated lymphocyte cultures suggest this possibility. However, the impaired DNA synthesis and carbohydrate metabolism may reflect other cellular defects induced by hyperoxia. These might include damage to one or more of the biochemical pathways required for normal cellular proliferation.

Presumably, the damage to lymphocytes caused by hyperoxia is mediated by the enhanced intracellular and extracellular production of toxic oxygen metabolites from oxygen. These would include an enhanced production of superoxide, hydrogen peroxide and hydroxyl free radical.[117] These are the same reactive oxygen species known to be generated by phagocytic cells during a metabolic burst. The relatively small increase in PO_2 (150 to 225 mm Hg) required to impair the proliferation of mitogen-stimulated human lymphocytes suggests that these cells are particularly sensitive to oxygen damage and indicates that lymphocytes may have suboptimal concentrations of cellular antioxidants and/or the protective enzyme system required to degrade ROS rapidly, particularly in sensitive cellular sites. The mammalian cell is known to have several mechanisms that normally protect it against oxidant damage by ROS. These defense mechanisms include the cellular enzymes superoxide dismutase, catalase, and glutathione peroxidase.[117–127] This latter enzyme requires glutathione as a cofactor, and is linked to the hexose monophosphate shunt pathway by the enzyme glutathione reductase. In addition, the mammalian cell has several antioxidants such as vitamins E and C. Lymphocytes have similar concentrations of superoxide dismutase, glutathione peroxidase, and glutathione reductase, compared with other blood cells.[120,121] However, lymphocytes have low concentrations of catalase which may relate directly to their sensitivity to oxidant damage.[122] It is clear that glutathione and glutathione peroxidase are important in cellular protection against oxidant damage to ROS. Cells deficient in glutathione or its related enzymes have increased sensitivity to oxidant damage even in cases in which there is an adequate cellular concentration of catalase.[118,119,124,125] However, the glutathione system may not be sufficient to protect the cell against H_2O_2 injury in the absence of cellular catalase, particularly in situations in which there is significant compartmentalization of these enzymes in the cell.

A second reason for the increased sensitivity of lymphocytes to oxidant damage may be related to a low cellular concentration of vitamin C. Vitamin C has

the capacity to degrade a number of ROS rapidly, including OH·.[120] Lymphocytes have been reported to take up less vitamin C from the extracellular medium and to have decreased capacity to reduce dehydroascorbate compared with other blood cells.[120]

In summary, the effects of hyperoxia on the proliferation of human lymphocytes demonstrate three points: (1) lymphocytes appear sensitive to oxidant damage, (2) oxidant damage under these conditions does not induce cell death but rather nonlethal cellular damage, and (3) these observations suggest that a relatively minor increase in the release of ROS by phagocytic cells at an inflammatory site might suffice to impair lymphocyte function.

3.2. Role of ROS in Radiation Damage to Lymphocytes

Human lymphoid cells appear to be relatively sensitive to damage by radiation compared with other blood cells and have been demonstrated to have impaired function following low-dose irradiation in vitro.[128–132] A similar impairment in function has been reported in the blood lymphocytes of patients who have received radiation therapy.[133–135] The mechanism of radiation damage to mammalian cells is complex and appears to involve multiple mechanisms of damage to the cell that can be modified by several repair mechanisms.[136–148] It has been established for some time that oxygen enhances the radiation damage occurring in most cells, including those of mammals—the oxygen-enhancing ratio (OER).[136–140,147,148] This OER is variable from tissue to tissue. The mechanism for the OER is unclear. This OER may be mediated by oxygen itself due to the formation of peroxy radicals[136] (see Chapter 12, this volume, concerning the Howard Flanders model of oxygen mediated radiation damage). Alternatively, it has been suggested that this oxygen-related damage may be mediated by O_2^-, H_2O_2, or additional OH· generated by a Haber–Weiss mechanism.[137–140,144–148] This point is currently controversial and the subject of considerable study.

The importance of oxygen in the sensitivity of blood lymphocytes to radiation damage is illustrated in Fig. 2. Following low-dose irradiation in vitro, human blood mononuclear cells display impaired lymphoblastic transformation to nonspecific mitogens. As shown, the sensitivity of human lymphocytes to damage by low-dose irradiation was enhanced markedly by oxygen. This observation indicates that either oxygen itself or a ROS produced from oxygen during radiation has the capacity to cause major damage to the lymphocyte.

The sensitivity of the lymphocytes to this oxygen-mediated radiation damage, similar to their sensitivity to oxidant damage under other conditions, may also relate to a decreased capacity of these cells to rapidly degrade ROS.[120]

There is evidence that GSH may protect complex cells against radiation damage.[147–149] This possibility has been raised by a number of observations. One is the study of the effects of radiation on bacteria and human fibroblasts deficient in GSH.[123,149] These mutant cells are equally sensitive to radiation under anaerobic and aerobic conditions and the degree to damage under both conditions is similar to

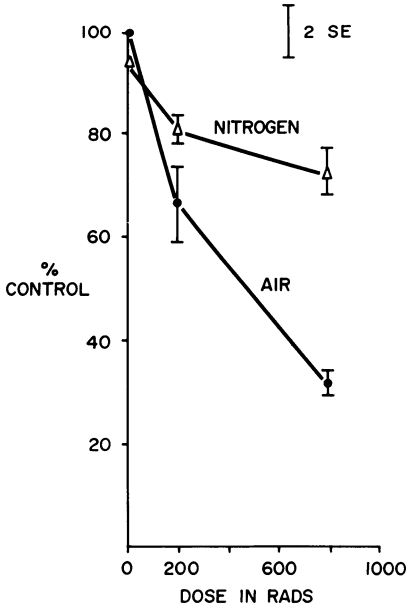

FIGURE 2. Comparison of lymphoblastic transformation to PHA after the irradiation of human lymphocyte cultures under an air and nitrogen atmosphere. DNA synthesis was quantitated by the [^{3}H]thymidine method. Results are expressed as uptake of unirradiated air control. (From Roberts et al.[130])

that occurring to the normal cells under oxygen. In addition, a number of studies indicate that the sensitivity of mammalian cells to radiation injury can be changed by modifying their sulfhydryl content.[123,147,148] In general, increasing the cellular concentrations of sulfhydryls is associated with resistence, while agents that deplete cellular sulfhydryl compounds appear to enhance sensitivity. In some cases, depletion of cellular GSH causes a substantial reduction in the OER. In other cases, GSH depletion is associated with sensitization of both aerated and hypoxic cultures resulting in no net change in the OER. Overall, the effect of sulfhydryl depletion may depend on the cell type used for study.

In this regard, GSH may also protect the lymphocyte against radiation damage. Diamide, an agent that rapidly oxidizes GSH, enhances the sensitivity of lymphocytes (Fig. 3) to radiation damage under hypoxic conditions but not aerobic conditions.[150] Similar to other tissues, the mechanism by which GSH protects the lymphocyte against radiation damage is unclear but may relate to its capacity to reduce organic radicals formed by OH·.[130,150]

3.3. Effect of Chemical Oxidation on Lymphocyte Function

About the same time that Anderson demonstrated that mild hyperoxia impairs the proliferative response of lymphocytes to nonspecific mitogens, several groups reported that human lymphocytes could be induced to proliferate by periodate, a potent oxidizing agent.[151–154] This activation may be secondary to oxidation of the terminal sialic acid of membrane glycoproteins or glycolipids to an aldehyde, result-

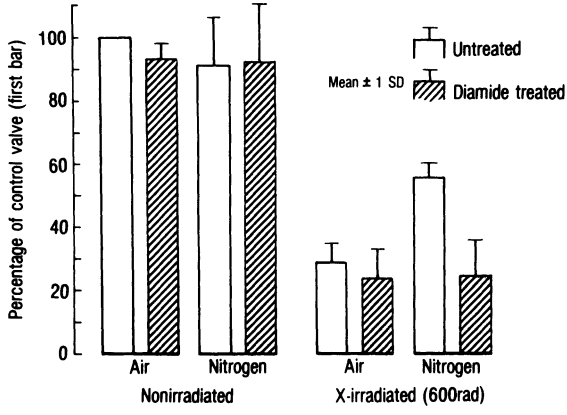

FIGURE 3. Effect of diamide and irradiation on the lymphoblastic transformation (LBT) of human lymphocyte cultures to PHA. The [^{3}H]Thymidine uptake during the final 4 hr of the 72-hr culture was used to quantitate DNA synthesis. Bars indicate mean ±SD of three experiments. Experimental values are expressed as percentage uptake of the unirradiated control under air (bar 1). The LBT of unirradiated lymphocyte cultures preincubated under hypoxic conditions (bar 3) were similar to the controls preincubated under air (bar 1). These control values range from 4–5 × 10^4 cpm/0.2 × 10^6 lymphocytes. Preincubation of lymphocyte suspensions with diamide under air (bar 2) or hypoxic conditions (bar 4) did not impair LBT. Following X-irradiation (600 rad) in an air atmosphere, diamide-treated suspension (bar 6) had an impairment in LBT similar to that in the irradiated suspensions without diamide (bar 5). Suspensions irradiated under hypoxic conditions were protected (bar 7). However, suspensions incubated with diamide under nitrogen (bar 8) had an impairment in LBT similar to suspensions incubated under air (bars 5 and 6). (From Sagone.[150])

ing a cellular signal for mitogenesis.[151] Periodate-induced proliferation requires careful control of the experimental conditions and is favored by low temperature, low concentrations of periodate, a short incubation time, and low pH. The excessive oxidation of the membrane by periodate results in a decreased response.[151] A similar induction of proliferation occurs following the incubation of lymphocytes with neuraminidase and galactose oxidase.[151] This treatment also results in the production of membrane aldehydes from galactose. Agents that react with aldehydes impair the response.[152] Therefore, it seems well established that membrane aldehydes are involved in chemical mitogenesis. Chemical mitogenesis is associated with the production of interleukin-2 (IL-2) and the induction of IL-2 receptors on sensitive T cells.[155] Therefore, this process appears to be analogous to the proliferation induced by nonspecific mitogens. Other lymphocyte functions might be similarly activated. It has also been reported that oxidation of cells with periodate or neuramindase–galactose oxidase induces enhanced lymphocyte cytotoxicity.[151]

Overall, the results of these experiments suggest that under some circumstances mild oxidation of the glycoconjungates on the membrane of lymphocytes might induce enhanced lymphocyte function in vivo. Chemical mitogenesis appears to represent altered lymphocyte function as a consequence of an extracellular oxi-

dant reacting primary with the cell membrane. The type of oxidant stress in system therefore is different from that occurring under hyperoxic conditions or as a consequence of radiation in which enhanced amounts of oxidants may be generated both intracellularly or extracellularly.

3.4. Effects of the ROS Generated by Enzyme Systems on the Functional Capacity of Lymphocytes in Vitro

Lymphocytes may be exposed to high fluxes of several ROS during an immunological reaction in vivo. Under these conditions, the lymphocyte would be exposed to oxidants generated primarily outside the cell. Theoretically, this situation would favor the interaction of the ROS with the membrane but, in some cases, particularly under conditions in which O_2^-, H_2O_2, or chloramines might be present in a high extracellular concentrations, significant amounts of the ROS could reach sensitive intracellular sites, causing oxidant damage. Under these conditions, damage could occur to the membrane and/or sensitive intracellular sites. Several methods have been used to induce oxidant damage to lymphocytes in vitro. These include xanthine oxidase enzyme system, the glucose–glucose oxidase enzyme system, the H_2O_2–myeloperoxidase enzyme system, and H_2O_2. This approach has permitted direct evaluation of the capacity of ROS to damage lymphocytes in the absence of other factors that may be released by phagocytic cells during cell–cell interaction. The results of these studies indicate that ROS impair several of the functions of lymphocytes and are discussed below.

3.4.1. Effect of Oxidant Damage on the Proliferation of Lymphocytes to Nonspecific Mitogens

Shortly after the reports that hyperoxia and chemical oxidation altered lymphocyte proliferation, we became interested in the possible effects of the ROS generated by phagocytic cells on the functional capacity of normal blood human lymphocytes.[156] Initially, we chose to study the effect of the ROS generated by the xanthine–xanthine oxidase system (X–XO) on the lymphoblastic transformation of human lymphocyte to nonspecific mitogens (PHA). This enzyme system seemed ideal, since it had already been reported to generate a sustained release of three of the ROS generated by phagocytic cells (O_2^-, H_2O_2, and OH·) and appeared analogous to the physiology of the metabolic burst known to occur in phagocytic cells.

The lymphoblastic transformation of mononuclear cell cultures was significantly impaired and delayed compared with controls following incubation with the X–XO enzyme system (Fig. 4). To identify the ROS causing the damage, we studied the protective effects of several agents that are scavengers of reactive oxygen species. Cultures supplemented with catalase were almost completely protected against the effect of the enzyme system. By contrast, cultures supplemented with superoxide dismutase and mannitol scavengers of O_2^- and OH·, respectively, were not. Therefore, the oxidant damage to the lymphocytes was mediated pri-

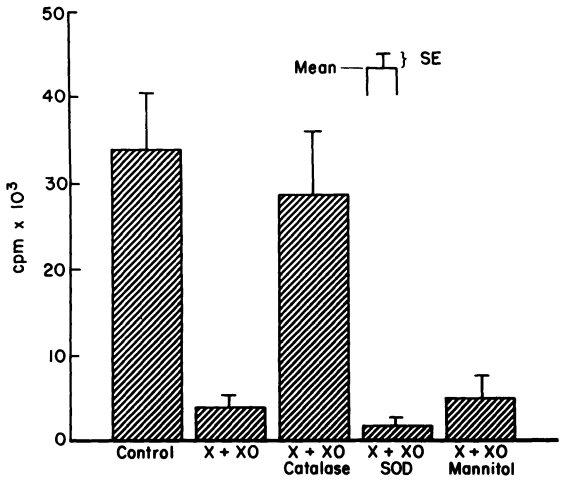

FIGURE 4. Effect of the ROS scavengers catalase, SOD, and mannitol on the transformation of lymphocyte cultures exposed to xanthine (X) and xanthine oxidase (XO). Bar 1 indicates the transformation of cultures incubated with PHA alone (controls). Bar 2 indicates cultures incubated with the PHA and the enzyme system. The remaining cultures were supplemented with catalase (bar 3), SOD (bar 4), or mannitol (bar 5). The scavengers alone did not alter the proliferation of the cultures in the concentrations used. (From Sagone et al.[156])

marily by H_2O_2 and not by the O_2^- or the OH· generated by the enzyme system. Recently, Zoschke and Messner[46] reported a similar impairment of LBT to concanavalin A following the incubation of human blood mononuclear cell with the X–XO enzyme system. They also concluded that H_2O_2 was the ROS mediating most of the damage. However, they also believed that OH· might be related to some of the damage, since ethanol as well as catalase was protective.

The impaired LBT cannot be explained by a decreased cell viability since the carbohydrate metabolism of unstimulated cultures exposed to the X–XO enzyme system was similar to controls[156] (Fig. 5). However, PHA-stimulated cultures did not have the augmented carbohydrate metabolism following H_2O_2 injury characteristics of normal cultures. This abnormality is similar to the impaired metabolism noted in PHA-stimulated lymphocyte cultures under hyperoxia (see Section 3.1).

We also studied the capacity of the H_2O_2 generated by the X–XO oxidase enzyme system to permeate the lymphocyte. We demonstrated that there was a marked stimulation of the HMPS shunt activity of lymphocytes during incubation with the enzyme system indicating the active degradation of H_2O_2 by glutathione peroxidase (Fig. 6). The addition of catalase to the incubation prevented the augmented HMPS activity. This experiment confirmed that H_2O_2, and not another ROS produced by the enzyme system, was related to the stimulation. This observation also provides solid evidence that a significant intracellular influx of H_2O_2 and presumably O_2^- was occurring under these experimental conditions. By contrast, purified lymphocyte cultures incubated with X–XO enzyme system oxidized only

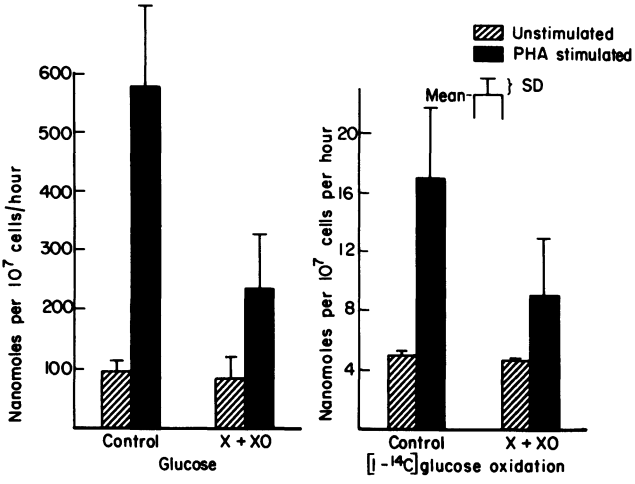

FIGURE 5. Effect of oxidant injury on the glucose metabolism of unstimulated and PHA-stimulated lymphocyte cultures. The mean glucose consumptions and [1-^{14}C]glucose oxidations of the cultures over the 3 days of incubation are given at the left and right, respectively. The values for the unstimulated cultures (three paired experiments) are indicated by the hatched bars and the values for mitogen-stimulated cultures (five paired experiments) by the solid bars. There is no significant difference in the rate of glucose consumption ($p > 0.2$) or [1-^{14}C]glucose oxidation ($p > 0.5$) in the unstimulated cultures incubated with the xanthine–xanthine oxidase (X–XO) enzyme system compared with the controls. The rate of glucose consumption ($p < 0.01$) and [1-^{14}C]glucose oxidation ($p < 0.05$) of the PHA-stimulated culture, incubated with the X–XO enzyme system, are significantly lower than the corresponding values of the PHA-stimulated controls. Values for the PHA-stimulated cultures are significantly higher than those of the corresponding unstimulated cultures. (From Sagone et al.[156])

small amounts of formate, suggesting little H_2O_2 degradation by intracellular catalase. Furthermore, the addition of azide to the cultures enhanced oxidant damage, suggesting an importance of intracellular catalase in the protection of the blood mononuclear cells against oxidant damage.

Other methods have been used to induce oxidant damage to lymphocytes in addition to the X–XO enzyme system. These include the addition of hydrogen peroxide to lymphocyte cultures,[41] the production of H_2O_2 by the glucose–glucose oxidase system,[46] H_2O_2 produced by the interaction of penicillamine and ceruloplasmin or copper,[157] and the H_2O_2–myeloperoxidase enzyme system.[158] In the latter system, both H_2O_2 and H_2O_2 generated by the glucose–glucose oxidase system have been used as a source of substrate. In all cases, H_2O_2 impairs the proliferation of lymphocytes to nonspecific mitogens.

In summary, H_2O_2 impairs the capacity of human lymphocytes to proliferate in response to nonspecific mitogens in a similar manner to hyperoxia (see Section 3.1) and by a mechanism that does not involve the significant death of the cell. It is of interest that this damage to lymphocytes occurs in spite of active intracellular

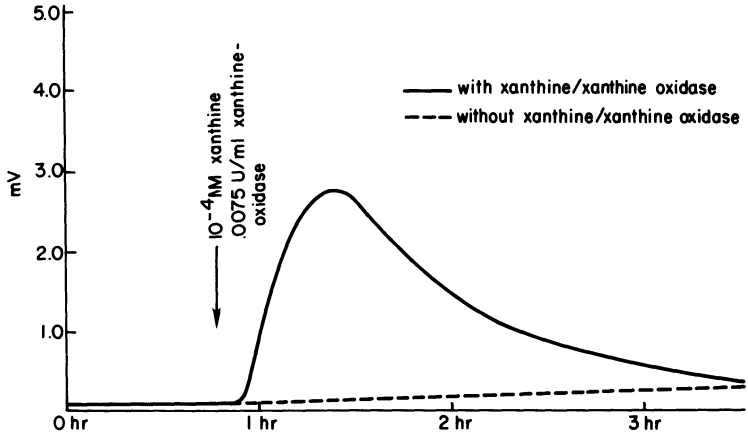

FIGURE 6. Effect of the xanthine and xanthine oxidase on the oxidation of [1-^{14}C]glucose by lymphocytes in short-term culture. The curves represent a continuous measurement of $^{14}CO_2$ production by lymphocyte suspensions in buffer using the ionization chamber electrometer system. The curves were drawn using the data points from a single experiment and are representative of four experiments performed. The *y* axis indicates the electric signal (in mV) generated by $^{14}CO_2$ in the ionization chamber. Signal generated by: untreated suspensions (- - -); suspensions supplemented with xanthine and xanthine oxidase (—). As indicated by the arrow, the addition of the enzyme system resulted in a prompt but transient increase in the oxidation of [1-^{14}C]glucose. The addition of substrate or the enzyme alone did not effect the oxidation of [1-^{14}C]glucose. (From Sagone et al.[156])

degradation of the H_2O_2 by the HMPS pathway of these cells and may relate to a low concentration of catalase in these cells.

3.4.2. Effect of ROS on the NK Activity and ADCC of Lymphocytes

Other studies have demonstrated that H_2O_2 similarly impairs other lymphocyte functions. These include the NK activity of lymphocytes against tumor target cells as well as their capacity to kill antibody-sensitized tumor cells (ADCC).[45,159–160] Also, H_2O_2 impairs the Staphylococcus protein A induction of human B-cell colony formation.[161] The concentrations of H_2O_2 required to impair these latter functions are in the range of 0.1 mM.[45,159–161] It is of interest that similar concentrations of H_2O_2 do not impair the capacity of monocytes to mediate ADCC suggesting that lymphocytes are more sensitive to oxidant damage than monocytes.[41] Furthermore, it has been suggested that the capacity of monocytes to rapidly degrade H_2O_2 may protect lymphocytes against oxidant damage.[157,158] This seems consistent with the observation that monocytes have a much higher concentration of catalase than lymphocytes.[122]

There is also evidence that H_2O_2 damage to T and B lymphocytes is associated with membrane alteration and/or impair microtubular function. These cells lose

their capacity to bind sheep RBC (rosette assay), have a decreased percentage of cells with surface IGG and have impaired Con A capping following H_2O_2 injury.[162]

3.4.3. Possible Role of the Myeloperoxidase Enzyme System in Oxidant Damage to Lymphocyte

The H_2O_2–myeloperoxidase system has been used to induce oxidant injury to lymphocytes. This physiological enzyme system serves an important role in the capacity of phagocytic cells, particularly granulocytes, to kill microorganisms.[159,163] Recent studies indicate that the ROS produced by this enzyme system may also cause oxidant damage to a variety of target tissues.[159] These include endothelial cells, tumor cells, platelets, and RBC.[159,164,165] In addition, HOCl and chloramine have the capacity to inactivate or activate extracellular enzymes.[166,167] Under some circumstances, myeloperoxidase may be released from phagocytic cells in vivo. Therefore, significant concentrations of HOCl or the stable oxidants derived from HOCl (chloramine) might develop at an inflammatory site.[21–23] Either of these reactive species potentially could induce altered lymphocyte function.

Recently, El-Hag and Clark and co-workers studied the capacity of the H_2O_2 myeloperoxidase enzyme system to impair lymphocyte functions.[158,159] They found that the addition of myeloperoxidase significantly reduces the concentration of H_2O_2 required to induce oxidant damage to both T and B lymphocytes. These observations suggest the possible importance of this enzyme system in oxidant damage to lymphocytes during cell–cell interaction.

3.5. Possible Differences in the Sensitivity of T and B Cells to Oxidant Damage

A number of studies have suggested that T and B cells may have a different sensitivity to oxidant damage.[158,168] Furthermore, some subsets of T cells, such as suppressor cells, may be particularly sensitive.[132] Recent studies by El-Hag et al.[158] have compared the sensitivity of a variety of T- and B-cell function to oxidant damage. These workers studied the effect of H_2O_2 injury with and without myeloperoxidase on the NK activity of lymphocytes, the proliferation of lymphocytes to the nonspecific mitogens and the generation of immunoglobulins secreting cells by pokeweed. All these lymphocyte functions could be impaired by oxidant injury. In all cases, the injury was greatest when the cultures were incubated with both H_2O_2 and MPO. However, similar results were found with H_2O_2 alone when the concentration used in the cultures was high enough. The formation of antibody-secreting cells to pokeweed proved most sensitive, while the proliferative response to pokeweed mitogen was found most resistant. The sensitivity of the lymphocyte proliferative responses to PHA and Con A as well as NK appeared to be intermediate. El-Hag et al. suggested the following rank order of sensitivity: B (or T helper) $>$ NK $\geq$ T. These results suggest that there may be a range of sensitivities of T- and B-lymphocyte subpopulations to oxidant damage. The complex nature of these

systems, however, makes it difficult in some cases to identify the precise mechanism involved in the altered response.

4. EVIDENCE THAT THE PRODUCTION OF ROS BY PHAGOCYTIC CELLS CAN ALTER LYMPHOCYTE FUNCTION

The results generated in the model systems discussed in Section 3 provide relatively solid evidence that oxidants may modify lymphocyte function. However, the obvious question is whether similar impairment of lymphocyte function occurs during cell–cell interaction in vivo in patients with a variety of diseases. The studies discussed in this section suggest that this is a likely possibility.

4.1. Evidence That the Release of ROS by Phagocytic Cells May Impair the NK Activity of Lymphocytes

A subset of T lymphocytes have the capacity to selectively recognize and kill target cells (NK activity)[169] and may therefore play an important role in the primary host defense, particularly against tumors, and infections. Similarly, a subset of lymphocytes is able to mediate cytotoxicity against the antibody-sensitized target cells (ADCC).[170] In 1981, Seaman et al.[169] reported that the NK activity of human blood mononuclear cells could be suppressed by the phorbol diester tumor promoters. The suppression of NK activity by these agents correlated with their potency as tumor promoters. The suppressive effect could be reduced if peripheral blood mononuclear cells (PBMC) were depleted of adherent cells indicating the importance of the monocyte in the suppressor activity.[169] In a subsequent study,[45] the same group evaluated the role of the ROS generated by monocytes as a mechanism for this suppressor activity. The NK activity of PMBC against the K 562 cells was decreased following incubation with (12-*o*-tetradecanoylphorbol-13-acetate) (TPA) or opsonized zymosan. Both agents stimulated the production of O_2^- and H_2O_2 by mononuclear cells. When PBMC were depleted of adherent cells, both the production of ROS and suppression of NK activity were virtually ablated. The activity could be restored by adding back either monocytes or granulocytes to the incubation. By contrast, the addition of granulocytes of patients with chronic granulomatous disease (CGG) that lack the capacity to generate ROS did not restore the suppressive effects. Scavenger studies were then done to identify the ROS primarily related to the damage. Catalase provided the most protection, while scavengers of OH· (mannitol, ethanol), were ineffective. Some protective effect of SOD was also observed. Azide, cyanide, and aminotriazole were also studied to evaluate the possible role of myeloperoxidase in the reaction. None of these agents provided a protective effect, and in fact the suppresion was enhanced in the experiments with zymosan. Therefore, MPO activity did not appear to be essential for the suppressor activity. It remains unclear what role the MPO pathway may play in the ROS

induced injury to lymphocytes, even though injury by the H_2O_2 of MPO enzyme has been shown to impair several lymphocyte function (see Section 3.4). Furthermore, the possibility that the stable oxidants produced from this system (chloramines) may have the capacity to induce oxidant damage has, as yet, not been the subject of extensive study. This seems an important point since the half life of these ROS at an inflammatory site may be much longer than the other ROS generated by phagocytic cells.[21–23]

There are other reports confirming that stimulated phagocytic cells may mediate suppressor cell activity against lymphocyte NK.[171] In some studies, nonoxidative mechanisms have also been suggested for the phagocytic cell-mediated suppression of NK or ADCC activity.[172,173] This latter observation is consistent with other reports indicating that monocytes and granulocytes have two mechanisms for mediating cytotoxicity.[93,95,98,99,174]

The capacity of TPA to suppress the NK activity of PBMC has led to one additional area of investigation, the capacity of TPA to stimulate the NK activity of purified lymphocyte cultures.[171] Under these conditions, lymphocytes incubated with PMA have increased NK activity after approximately 24 hr in culture. The capacity of PMA to increase an activity is synergistic with other agents known to induce the NK activity of lymphocytes such as IFN or IL-2.

4.2. Evidence That ROS Released by Phagocytic Cells Can Alter Lymphocyte Proliferation

4.2.1. Experiments in Which Phagocytic Cells Have Been Stimulated to Release ROS during Coculture with Lymphocytes

Several recent studies have established that the ROS released by monocytes and granulocytes suppress human lymphocyte mitogenesis.[41,46,175] Zoschke and Messner studied the capacity of monocyte and granulocytes stimulated with a variety of agents to suppress lymphocyte mitogenesis to Con A. The agents studied included PMA, the chemotactic peptide *n*-formyl L-methionyl-leucyl,phenylalanine (FMLP), opsonized zymosan particles, and heat-aggregated γ-globulin.

The results with PMA are of particular interest, since this compound is known to be a potent stimulator of the oxidation metabolism of phagocytic cells (see Fig. 1), as well as a mitogen for human lymphocytes. This is not true of other mitogens such as PHA, Con A, and pokeweed mitogen. The PMA induced transformation of human blood mononuclear cells containing 20% monocytes and 80% lymphocytes could be augmented by the addition of catalase to the cultures, indicating that the H_2O_2 released by the monocytes could suppress mitogenesis. By contrast, the addition of scavengers of O_2^- and hydroxyl radical had no effect. As expected, the protective effect of catalase was not observed in PMA-stimulated lymphocyte cultures depleted of monocytes (<5%) confirming that the monocyte were related to the suppressor activity. In contrast to PMA cultures, there was no augmentation of LBT in Con A-stimulated cultures supplemented with catalase. This observation

confirms that Con A, similar to PHA, does not stimulate a significant metabolic burst or release of ROS in phagocytic cells. When PMA was added to Con A-stimulated cultures as a comitogen, the LBT was not greater than that observed with either mitogen alone. However, the addition of catalase to the cultures augmented their proliferation twofold, again indicating suppressor activity due to the release of H_2O_2 from monocytes. In this regard, the catalase had to be added early in the cultures to provide a protective effect. Similar results were found in experiments in which lymphocytes were cocultured with granulocytes rather than monocytes.

Suppression of LBT to Con A was also observed when lymphocyte–granulocyte cultures were stimulated with opsonized zymosan particles, FMLP, and aggregated immunoglobulin. Varying degrees of suppression were observed, depending on the stimulant used. Catalase added to the cultures again protected against this suppressive effect, although in some cases this protection was not complete. Finally, similar experiments were done using the blood cells of patients with CGD. As opposed to normals, the LBT of the lymphocyte cultures of CGD patients was not inhibited by PMA stimulation of their phagocytic cells. However, catalase-reversible suppression could be shown when normal phagocytic cells were cocultured with the lymphocytes of these patients.

We have also studied the capacity of the H_2O_2 released by PMA-stimulated blood mononuclear cell cultures to alter PMA-induced mitogenesis.[41] We found that the addition of catalase to PMA-stimulated cultures augmented their LBT (see Fig. 7). As expected, catalase did not alter the proliferation of mononuclear cell cultures stimulated by PHA, since the mitogen does not stimulate an immediate release of ROS.

Our measurements indicate the concentration of H_2O_2 reached values as high as 0.008 mM under our culture conditions. Since factor(s) released by monocytes also appears to augment lymphocytic transformation,[176] we evaluated the possibility that the monocyte might be autooxidized by its own ROS, thereby impairing its own function. However, we were unable to demonstrate impaired monocyte ADCC following incubation with 0.1 mM H_2O_2, a concentration known to ablate the NK and ADCC of lymphocytes.[160] Of interest is the observation that monocytes pretreated with azide and then exposed to H_2O_2 had a marked suppression of the ADCC activity (<90%). This observation suggests that catalase protects these cells against oxidant damage; it also supports the idea that the greater sensitivity of lymphocytes to oxidant damage is related to a low concentration of catalase in these cells.

Overall, the results of the experiments discussed above indicate that the H_2O_2 released by phagocytic cells directly injures one or more subpopulation(s) of lymphocytes required for optimal T-cell proliferation. However, the possibility that the effect of low fluxes of H_2O_2 can augment the function of some cells such as suppressor cells cannot be excluded. Also, the effects could be secondary to an altered function of the lymphokines required for LBT. Furthermore, it is possible that oxidant damage may be associated with multiple sites of injury.

Other studies have documented that oxidant damage impairs B-cell function.

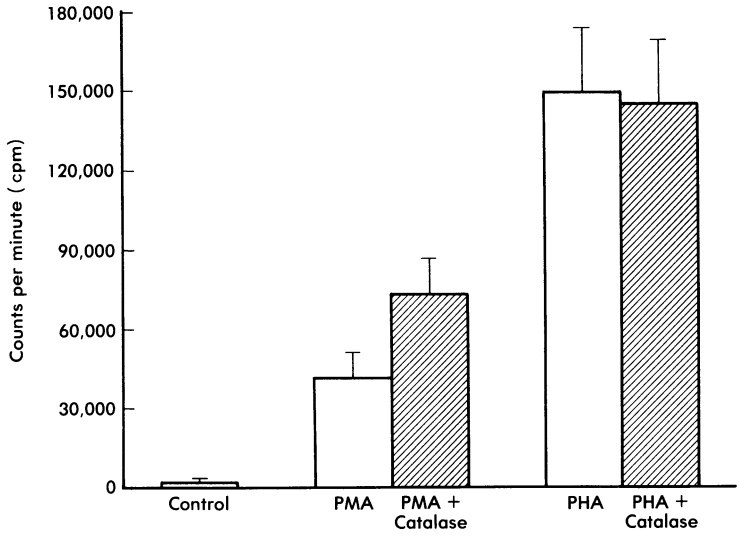

FIGURE 7. Effect of catalase on the LBT of mononuclear cells to PMA and PHA. The *y* axis indicates the thymidine uptake of the cultures (in cpm). The results indicate the mean ±1 SD for four paired experiments. The mean value for cultures with PMA is significantly lower than that for PMA-treated cultures supplemented with catalase $p < 0.02$). (From Sagone et al.[41])

Whisler and Newhouse[161] reported that the endogenous release of H_2O_2 as well as prostaglandins by mononuclear cells inhibited the formation of human B-cell colonies stimulated by protein Staph A. Also, the inhibition of colony formation following the addition of lipopolysaccharide (LPS) to the cultures could be reversed by the addition of catalase or indomethacin. These observations indicate that the H_2O_2 and prostaglandins released by monocytes were related to the LPS-induced suppressive effect. These results provide evidence that the amount of H_2O_2 released by phagocytic cells is also sufficient to alter B-cell function. In both cases, the stimulus (protein Staph A and LPS) probably induce the direct release of H_2O_2 by the monocytes.

Archibald et al.[100] recently demonstrated that an antigen–antibody complex—bovine serum albumin (BSA) with anti-BSA—or aggregated γ-globulin bound to B lymphocytes triggers a respiratory burst in neutrophils. This is indicated by the generation of chemiluminescence by the PMN during coculture with the lymphocytes. These investigators also demonstrated that the lymphocytes had an impaired proliferation to pokeweed mitogen but not to PHA mitogen after periods of coculture with the granulocytes. The impaired response could not be explained by decreased viability of the cells. This is an interesting system in which the lymphocytes trigger the PMN to release ROS, which then impair the functional capacity of the lymphocytes. The authors suggest that the stimulation of PMNs by lymphocyte

surface-bound immune complexes might lead to similar lymphocyte damage in vivo.

4.2.2. Studies That Have Evaluated the Effect of the Spontaneous Release of ROS by Phagocytes on Lymphocyte Proliferation

Numerous recent reports have established the importance of ROS in the functional capacity of activated macrophages, particularly in their bacterial and tumoricidal activities. These cells are also known to have the capacity to suppress lymphocyte function.[177–180] Similarly, the blood monocytes and granulocytes of patients with inflammatory and malignant disorders have been reported to have activated oxidative metabolism and function.[175,181–186] Presumably, this activation is associated with the release of ROS in vivo and suggests that the release of ROS by activated phagocytes might be one mechanism for the suppression of lymphocyte function found in several groups of patients. In this regard, several recent studies have evaluated the possible role of ROS in the suppressor cell activity of activated phagocytic cells against lymphocytes.

Metzger et al.[179] in 1980 reported that peritoneal exudate cells (PEC) induced by *Corynebacter parvum* or thioglycolate could suppress the proliferation of murine splenic lymphocytes to Con A in vitro.[179] At 2 : 10 ratio, the macrophages totally suppressed lymphocyte transformation. At a lower macrophage-to-lymphocyte ratio, partial suppression occurred (60–68%). Partial suppression of approximately 50% could be demonstrated in cultures incubated with prostaglandins E_1 and E_2 (PGE_1 and PGE_2) in high concentrations (10^{-8}–10^{-5} M). The suppression did not occur with similar concentrations of $PGF_{2\alpha}$. These concentrations of prostaglandins were higher than those generated by the PEC during cocultures with the lymphocytes. Therefore, the release of prostaglandins from the PEC did not entirely explain the suppressive effect on lymphocyte function. The possible role of H_2O_2 and of prostaglandins in this suppressor cell activity was then evaluated by adding catalase and indomethacin to the cultures. The addition of either indomethacin or catalase could protect the partially suppressed cultures. These agents were relatively ineffective, however, in protecting the totally suppressed cultures. The simultaneous addition of catalase and indomethacin to these cultures provided complete protection against macrophage-mediated suppression. These experiments suggest that the suppressive effects of PEC against lymphocytes was mediated by both prostaglandins and H_2O_2. While the precise mechanism involved in the protective effects of catalase and indomethacin in this system is not entirely clear, the experiments provide evidence that H_2O_2 may be involved in the suppressor activity of macrophages in vivo and seem to approximate conditions in vivo in which lymphocytes would be exposed chronically to H_2O_2.

It has been established for some time that patients with Hodgkin's disease have impaired cellular immunity.[180,187,188] These patients have impaired skin reactivity, and their blood lymphocytes have impaired proliferation following stimulation by

nonspecific mitogens in vitro. Recent studies evaluated the possibility that this impaired lymphocyte function might be due to the release of H_2O_2 by the monocytes in the cultures.[180,187] However, while the results of these studies are of interest, they proved to be complex.[180,187] As expected, the blood mononuclear cells (PMBC) of most Hodgkin's disease patients had an impaired proliferation to PHA. When the PBMC were depleted of monocytes, the proliferation was enhanced indicating that monocytes were suppressing the response by some mechanism. When catalase was added to the cultures, no protective effect was observed, apparently excluding H_2O_2 as a major mediator of the suppressor activity. By contrast, the addition of indomethacin augmented the proliferation of the cultures in a similar manner to monocyte depletion, suggesting that release of prostaglandin by the monocytes was one mechanism for the suppression. Unexpectedly, the simultaneous addition of catalase and indomethacin further augmented the LBT of cultures, including those of normals as well as those from the patients. Overall, the response still remained significantly impaired in the patient cultures compared with those of normals. The failure of catalase and indomethacin to provide complete protection indicates that additional factor(s) besides prostaglandins and H_2O_2 relate to the impaired LBT of these cells. Fisher and Bostick-Bruton[180] concluded that the T cells of patients with Hodgkin's disease probably have an intrinsic defect as a major etiology for their impaired function. The mechanism for the enhanced response in cultures with catalase and indomethacin is unclear. One explanation for the effect may be in the capacity of prostaglandins to inhibit the production of H_2O_2 by monocytes.[189] While these studies do not establish a causal relationship between the release of ROS by monocytes and the impaired T-cell function of Hodgkin patients, they do provide additional evidence that the release of ROS by phagocytes may alter lymphocyte proliferation.

Thus far, the effects of the ROS released by activated granulocytes on the functional capacity of lymphocytes does not appear to have been the subject of extensive evaluation. Niwa et al.[175] recently reported that the stimulated neutrophils from the synovial fluid with rheumatoid arthritis can alter the function of lymphocytes. The release of ROS by the unstimulated and zymosan-stimulated granulocytes from the joints of these patients was enhanced compared with blood granulocytes of normal persons. The coculture of these activated granulocytes with patient and normal lymphocytes for 17 hr resulted in a decreased number of OKT_4^+ and OKT_8^+ cells and an impaired response to nonspecific mitogens. The lymphocytes could be protected by the addition of catalase and SOD, indicating that the suppressor effect was mediated by the release of ROS from these cells. These results demonstrate that the ROS released by activated granulocytes have the capacity to impair lymphocyte function.

4.3. Role of the Lymphocyte in the Metabolic Burst

Numerous reports indicate that lymphocytes, in contrast to phagocytic cells, lack the capacity to undergo a metabolic burst. There is some evidence, however,

that OH· may be involved in the functional capacity of these cells. Hydroxyl scavengers have been reported to inhibit the proliferation of human lymphocyte cultures to nonspecific mitogens.[79] The mitogenesis induced by PMA is much more sensitive to inhibition than that induced by PHA or Con A. Hydroxyl scavengers also impair the proliferation of mouse thymocyte cultures to IL-2.

These observations suggest that OH· is involved in some way in mitogenesis. The mechanism is not clear and needs further study. These studies are of interest, however, since they represent one of the few observations in the literature that indicate that oxidants may enhance lymphocyte function during cell–cell interaction.

Also, some reports indicated that OH· may be related to the NK activity of lymphocytes.[80] If so, these cells may have a burst in OH· production after conjugate formation with the target cell. This evidence is based primarily on scavenger studies and therefore represents indirect evidence for the production of OH· in this cell. However, recent reports have also suggested a role of the lipoxygenase pathway in NK activity.[190] This pathway has been shown to produce OH· in other cells,[191] so it is possible that OH· production could occur in the lymphocyte by this pathway as well. This explanation could link the role of the lipoxygenase pathway and OH· in the NK activity of lymphocytes.[190] Another possible source of OH· production in lymphocytes could be microsomes, which have been established to generate OH· (see Chapter 12). If lymphocytes do produce OH· under some circumstances, this ROS might alter the function of other cells, particularly some subpopulations of lymphocytes.[79]

Finally, activated lymphocyte subsets are known to produce a number of factors (lymphokines) such as interleukin, interferon, and peroxide stimulating factor that may activate both lymphocytes and phagocytic cells.[69–72] Such activated phagocytic cells, once stimulated, should release increased amounts of ROS and have increased suppressor cell activity. Whether such a feedback mechanism plays a physiological role in the immune response would seem to be of interest.

5. SUMMARY

The studies that have been discussed indicate that the ROS released by phagocytic cells can impair the functional capacity of blood T and B lymphocytes. This sensitivity of lymphocytes to H_2O_2 may relate to a low concentration of catalase and/or other antioxidants in these cells. The major ROS shown to cause injury in these in vitro systems has been H_2O_2, but it seems likely that the other ROS released by phagocytes might also impair lymphocyte function under some conditions in vivo. While chemical mitogenesis suggests that oxidants might augment lymphocyte function under some conditions, thus far, only a few reports indicate that this occurs during cell–cell interaction.

Presumably, impaired lymphocytes function might occur in patients with a variety of diseases. However, establishing a cause–effect relationship between the

release of ROS by phagocytic cells in vivo and an impaired lymphocyte function is more complicated. Phagocytic cells are known to have nonoxidative mechanisms as well as oxidative ones for injurying target cells. Therefore, the differentiation of oxidative and nonoxidative damage to lymphocytes by phagocytic cells may prove difficult. Also, other factors may alter lymphocyte function in vivo. These include prostaglandins, lymphokines, and serum factors, to mention a few. Furthermore, the situation may be even more complex in some groups of patients, such as those with cancer. Recently, neoplastic cells have been shown to release factors that impair the functional capacity of both lymphocytes and phagocytic cells.[192–194] In spite of the complex nature of the immune system, it seems likely that future studies will establish that the ROS generated by phagocytes have an important role in regulating the immune response in vivo.

REFERENCES

1. Klebanoff SJ: Antimicrobial mechanism in neutrophilic polymorphonuclear leukocytes. *Semin Hematol* **12:**117–142, 1975.
2. Babior BM: The respiratory burst of phagocytes. *J Clin Invest* **73:**559–601, 1984.
3. Johnston RB, Lehmeyer JE: Elaboration of toxic oxygen by-products by neutrophils in a model of immune complex disease. *J Clin Invest* **51:**836–841, 1976.
4. Babior BM, Kipnes R, Curnette JT: Biological defense mechanisms. The production of leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* **52:**741–744, 1973.
5. Johnston RB Jr, Keele BB, Misra HP, et al: The role of superoxide anion generation in phagocytic bactericidal activity—Studies with normal and chronic granulomatous disease leukocytes. *J Clin Invest* **55:**1357–1372, 1975.
6. Lehmeyer JE, Johnston RB Jr: Effect of anti-inflammatory drugs and agents that elevate intracellular cyclic AMP on the release of toxic oxygen metabolites by phagocytes: Studies in a model of tissue-bound IgG. *Clin Immunol Immunopathol* **9:**482–490, 1978.
7. Iyer GY, Islam MF, Quastel JH: Biochemical aspect of phagocytosis. *Nature (Lond.)* **192:**535–541, 1961.
8. Sbarra AJ, Karnovsky ML: The biochemical basis of phagocytosis I, Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J Biol Chem* **234:**1355–1362, 1959.
9. Karnovsky ML: Metabolic basis of phagocytic activity. *Physiol Rev* **42:**143–168, 1962.
10. Curnette JT, Babior B: Biological defense mechanisms, the effect of bacteria and serum on superoxide production by granulocyte. *J Clin Invest* **53:**1662–1672, 1974.
11. Homan-Muller J, Weening RS, Roos D: Production of hydrogen peroxide by phagocytosing human granulocytes *J Lab Clin Med* **85:**198–207, 1975.
12. Allen RC, Stjernholm R, Steele RH: Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. *Biochem Biophys Res Commun* **47:**679–684, 1972.
13. Webb LS, Keele BB Jr, Johnston RB Jr: Inhibition of phagocytosis-associated chemiluminescence by superoxide dismutase. *Infect Immun* **9:**1051–1056, 1974.
14. Baehner RL, Murrman SK, Davis J, et al: The role of superoxide anion and hydrogen peroxide in phagocytosis-associated oxidative metabolic reactions. *J Clin Invest* **56:**571–576, 1975.
15. Sagone AL Jr, King GW, Metz EN: A comparison of the metabolic responses to phagocytosis in human granulocytes and monocytes. *J Clin Invest* **57:**1352–1358, 1976.
16. Klebanoff SJ, Pincus SH: Hydrogen peroxide utilization in myeloperoxidase-deficient leukocytes: A possible microbicidal control mechanism. *J Clin Invest* **50:**2226–2229, 1971.

17. Weening RS, Roos P, Loos JA: Oxygen consumption of phagocytizing cells in human leukocyte and granulocyte preparations: A comparative study. *J Lab Clin Med* **83:**570–576, 1974.
18. Root RK, Metcalf J, Oshino N: H_2O_2 release from human granulocytes during phagocytosis. I. Documentation, quantitation, and some regulating factors. *J Clin Invest* **55:**945–955, 1975.
19. Tsan Min Fu, Chen JW: Oxidation of methionine by human polymorphonuclear leukocyte. *J Clin Invest* **65:**1041–1050, 1980.
20. Weiss SJ, Klein R, Slivka A, et al: Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. *J Clin Invest* **70:**598–607, 1982.
21. Thomas EL, Grisham MB, Jefferson MM: Myeloperoxidase-dependent effect of amines on function of isolated neutrophils. *J Clin Invest* **72:**441–454, 1983.
22. Weiss SJ, Lambert MD, Test ST: Long-lived oxidants generated by human neutrophils: Characteristics and bioactivity. *Science* **222:**625–628, 1983.
23. Sagone AL Jr, Husney RM, O'Dorisio MS, et al: Mechanisms for the oxidation of reduced glutathione by zymosan stimulated granulocytes. *Blood* **63:**96–104, 1984.
24. Pincus S, Klebanoff SJ: Quantitative leukocyte iodination. *N Engl J Med* **284:**744–750, 1984.
25. McCord J: Free Radicals and inflammation: Protection of synovial fluid by superoxide dismutase. *Science* **185:**529–531, 1974.
26. Oxygen free radicals and tissue damage, in Fitzsimons DW (ed): *Ciba Foundation Symposium 65,* Excerpta Medica Amsterdam, New York, 1979, 1–381.
27. Weissman G, Smolen JE, Korchak EM: Release of inflammatory mediators from stimulated neutrophils. *N Engl J Med* **303:**27–34, 1980.
28. Weissman G, Serhan C, Korchak HM, et al: Neutrophils generate phosphatidic acid, an "endogenous calcium ionophore: Before releasing mediators of inflammation. *Trans Assoc Am Phys* **94:**357–365, 1981.
29. Boxer LA, Yoder M, Bonsib S, et al: Effect of a chemotactic factor, N-formylmethionyl peptide on adherence, superoxide anion generation, phagocytosis and microtubule assembly of human polymorphonuclear leukocytes. *J Lab Clin Med* **93:**506–514, 1979.
30. Koretzky GA, Daniele RP, Nowell PC: A phorbol ester (TPA) can replace macrophages in human lymphocyte cultures stimulated with a mitogen but not with an antigen. *J Immunol* **128:**1776–1780, 1982.
31. Simon PL, Willoughby WF: The role of subcellular factors in pulmonary immune function: Physicochemical characterization of two distinct species of lymphocyte-activating factor produced by rabbit alveolar macrophages. *J Immunol* **126:**1534–1541, 1981.
32. Goldstein IM, Roos D, Kaplan HB, Weissman G: Complement and immunoglobulin stimulate superoxide production by human leukocytes independently of phagocytosis. *J Clin Invest* **56:**1155–1163, 1975.
33. Henson PM, Oades ZG: Stimulation of human neutrophils by soluble and immunoglobin aggregates—Secretion of granule constituents and increased oxidation of glucose. *J Clin Invest* **56:**1053–1061, 1975.
34. Weksler BB, Goldstein IM: Prostaglandin: Interactions with platelets and polymorphonuclear leukocytes in hemostasis and inflammation. *Am J Med* **68:**419–428, 1980.
35. Segal ML, Fertel RH, Kraut EH, Sagone AL: The role of reactive oxygen species in thromboxane B_2 generation by polymorphonuclear leukocytes. *J Lab Clin Med* **102:**788–794, 1983.
36. Goldstein IM, Malmsten CL, Kindahl H, et al: Thromboxane generation by human neutrophils polymorphonuclear leukocytes. *J Exp Med* **148:**787–792, 1978.
37. Mallery SR, Zeligs BJ, Ramwell PW, Bellanti JA: Gender-related variations of human neutrophil cyclo-oxygenase and oxidative burst metabolites. *J Leukocyte Biol* **40:**133–148, 1986.
38. Sagone AL Jr, Decker MA, Wells RM, Democko C: A new method for the detection of hydroxyl radical production. *Biochim Biophys Acta* **628:**90–97, 1980.
39. Alexander MS, Husney RM, Sagone AL Jr: Metabolism of benzoic acid by stimulated polymorphonuclear cells. *Biochem Pharmacol* **35:**3649–3651, 1986.
40. Sagone AL, Husney RM: Oxidation of salicylates by stimulated granulocytes—Evidence that these drugs act as free radical scavengers in biological systems. *J Immunol* **138:**1–7, 1987.

41. Sagone AL, Husney R, Guter H, Clark L: The effect of catalase on the proliferation of human lymphocytes to phorbol myristate acetate. *J Immunol* **133:**1488–1494, 1984.
42. Repine JE, Eaton JW, Anders MW, et al: Generation of hydroxyl radicals by enzymes, chemicals, and human phagocytes in vitro. Detection with the anti-inflammatory agent, dimethyl sulfoxide. *J Clin Invest* **64:**1642–1651, 1979.
43. Klassen D, Conkling P, Sagone AL: Activation of monocyte and granulocyte antibody-dependent cytotoxicity by phorbol myristate acetate. *Infect Immun* **35:**818–825, 1982.
44. Papermaster-Bender G, Whitcomb M, Sagone AL Jr: Characterization of the metabolic responses of human pulmonary alveolar macrophage. *J Reticulendothel Soc* **28:**129–139, 1980.
45. Seaman WE, Gindhart TD, Blackman MA, et al: Suppression of natural killing in vitro by monocytes and polymorphonuclear leukocytes. *J Clin Invest* **69:**876–888, 1982.
46. Zoschke DC, Messner RP: Suppression of human lymphocyte mitogenesis mediated by phagocyte-released reactive oxygen species: comparative activities in normals and in chronic granulomatous disease. *Clin Immunol Immunopathol* **32:**29–40, 1984.
47. Pryzwansky KB, Martin LE, Spitznagel JK: Immunocytochemical localization of myeloperoxidase, lactoferrin, lysozyme and neutral proteases in human monocytes and neutrophilic granulocytes. *J Reticuloendothel Soc* **24:**295–309, 1978.
48. Zabos P, Kyner D, Mendelsohn N, et al: Catabolism of 2-deoxyglucose by phagocytic leukocytes in the presence of 12-*O*-tetradecanoyl phorbol-13-acetate. *Proc Natl Acad Sci USA* **75:**5422–5426, 1978.
49. Nathan CF, Brukner LH, Silverstein SC, et al: Extracellular cytolysis by activated macrophages and granulocytes. I. Pharmacologic triggering of effector cells and the release of hydrogen peroxide. *J Exp Med* **49:**84–99, 1979.
50. Murray HW, Juangbhanich CW, Nathan CF, et al: Macrophage oxygen-dependent antimicrobial activity. *J Exp Med* **150:**950–964, 1979.
51. Cohen MS, Taffet ST, Adams DO: The relationship between competence for secretion of H_2O_2 and completion of tumor cytotoxicity by BCG-elicited murine marcophages. *J Immunol* **128:**1781–1785, 1982.
52. Saito H, Tomioka H, Watanabe T: H_2O_2 releasing function of macrophages activated with various mycobacteria based on wheat germ agglutinin and phorbol myristate acetate triggering. *J Reticuloendothel Soc* **29:**193–204, 1981.
53. Pennline KJ, Herscowitz HB: Dual role for alveolar macrophages in humoral and cell-mediated immune responses: evidence for suppressor and enhancing functions. *J Reticuloendothel Soc* **30:**205–217, 1981.
54. Drath DB, Karnovsky ML, Huber GL: Hydroxyl radical formation in phagocytic cells of the rat. *J Appl Physiol* **46:**136–140, 1979.
55. Heifets L, Imai K, Goren MB: Expression of peroxidase-dependent iodination by macrophages ingesting neutrophil debris. *J Reticuloendothel Soc* **28:**391–404, 1980.
56. Hoidal J, Repine J, Beall G et al: The effect of phorbol myristate acetate on the metabolism and ultrastructure of alveolar macrophages. *Am J Pathol* **91:**469–476, 1978.
57. Murray H, Cohn Z: Macrophage oxygen-dependent antimicrobial activity III Enhanced oxidative metabolism as an expression of macrophage activation. *J Exp Med* **152:**1596–1609, 1980.
58. North RJ: Opinions: The concept of the activated macrophage. *J Immunol* **121:**806–816, 1978.
59. Turpin J, Hersh EM, Lopez-Berestein G: Characterization of small and large human peripheral blood monocytes: effects of in vitro maturation on hydrogen peroxide release and on the response to macrophage activators. *J Immunol* **136:**4194–4198, 1986.
60. Montarroso AM, Myrvik QN: Oxidative metabolism of BCG-activated alveolar macrophages. *J Reticuloendothel Soc* **25:**559–574, 1979.
61. Douglas SD: Macrophage nomenclature: Where are we going? *J Reticuloendothel Soc* **27:**241–245, 1980.
62. Breton-Gorius J, Guichard J, Vainchenker W, et al: Ultrastructural and cytochemical changes induced by short and prolonged culture of human monocytes. *J Reticuloendothel Soc* **27:**289–301, 1980.

63. Anderson SE Jr, Remington JS: Effect of normal and activated human macrophages on toxoplasma gondii. *J Exp Med* **139:**1154–1173, 1974.
64. Karnovsky ML, Simmons S, Glass EA: Metabolism of macrophages, in Van Furth R (ed): *Mononuclear Phagocytes* Philadelphia, F. A. Davis, 1970, p 103–120.
65. Musson RA, Shafran H, Henson PM: Intracellular levels and stimulated release of lysosomal enzymes from human peripheral blood monocytes and monocyte-derived macrophages. *J Reticuloendothel Soc* **28:**249–264, 1980.
66. Rosenthal AS: Medical intelligence. Current concepts: Regulation of the immune response—Role of the macrophage. *N Engl J Med* **303:**1153–1156, 1980.
67. Speert DP, Silverstein SC: Phagocytosis of unopsonized zymosan by human monocyte-derived macrophages: Maturation and inhibition by mannan. *J Leukocyte Biol* **38:**655–658, 1985.
68. Soler P, Basset F, Mazin F, et al: Peroxidatic activities of human alveolar macrophages in some pulmonary granulomatous disorders. *J Reticuloendothel Soc* **31:**511–521, 1982.
69. Gately CL, Wahl SM, Oppenheim JJ: Characterization of hydrogen peroxide-potentiating factor. A lymphokine that increases the capacity of human monocytes and monocyte-like cell lines to produce hydrogen peroxide. *J Immunol* **131:**2853–2858, 1983.
70. Chapes SK, Haskill S: Synergistic effect between neutrophils and corynebacterium parvum in the process of macrophage activation. *Cancer Res* **44:**31–34, 1984.
71. Weisbart RH, Kwan L, Golde DW: Human GM-CSF primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants. *Blood* **69:**18–21, 1987.
72. Nathan CF, Murray HW, Wiebe ME, et al: Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J Exp Med* **158:**670–689, 1983.
73. Wilson ME, Jones DP, Munkenbeck P, et al: Serum-dependent and independent effects of bacterial lipopolysaccharides on human neutrophil oxidative capacity in vitro. *J Reticuloendothel Soc* **31:**43–57, 1982.
74. Nakagawara, A, Desantis NM, Nogueira N, et al: Lymphokines enhance the capacity of human monocytes to secrete reactive oxygen intermediates. *J Clin Invest* **70:**1042–1048, 1982.
75. Sagone AL Jr, LoBuglio AT, Balcerzak SP: Alteration in hexose monophosphate shunt during lymphoblastic transformation. *Cell Immunol* **14:**443–452, 1974.
76. Hadden JW, Hadden EM, Good RA: Adrenergic mechanisms in human lymphocyte metabolism. *Biochim Biophysic Acta* **237:**330–347, 1971.
77. Roos D, Loos JA: Changes in the carbohydrate metabolism of mitogenically stimulated human peripheral lymphocytes. *Biochim Biophys Acta* **222:**565–582, 1970.
78. Stjernholm RL: Early biochemical changes in phytohemagglutinin-stimulated human lymphocytes of blood and lymph. *J Reticuloendothel Soc* **7:**471–483, 1970.
79. Novogrodsky A, Ravid A, Rubin A, et al: Hydroxyl radical scavengers inhibit lymphocyte mitogenesis. *Proc Natl Acad Sci USA* **79:**1171–1174, 1982.
80. Duwe AK, Werkmeister J, Roder JC: Natural killer cell-mediated lysis involves an hydroxyl radical-dependent step. *J Immunol* **134:**2637–2644, 1985.
81. Mookerjee BK, Wakerle H, Sharon N, et al: Chemiluminescence and lymphocyte proliferation: parallelism in collaboration between subpopulations of thymus cells for both types of responses. *J Leukocyte Biol* **35:**427–438, 1984.
82. Kurlander RJ, Rosse WF, Logue GL: Quantitative influence of antibody and complement coating of red cells on monocyte-mediated cell lysis. *J Clin Invest* **61:**1309–1319, 1978.
83. Adler R, Glorioso JC, Cossman J, et al: Possible role of Fc receptors on cells infected and transformed by herpesvirus: Escape from immune cytolysis. *Infect Immun* **21:**442–447, 1978.
84. Ehlenberger AG, Nussenzweig V: The role of membrane receptors for C3b and C3d in phagocytosis. *J Exp Med* **145:**357–371, 1977.
85. Wuest D, Crane R, Rinehart JJ: Enhancement of Fc receptor function during human monocyte differentiation in vitro. *J Reticuloendothel Soc* **30:**147–155, 1981.
86. Newman SL, Becker S, Halme J: Phagocytosis by receptors for C3b (CR_1), iC3b, (CR_3), and IgG (Fc) on human peritoneal macrophages. *J Leukocyte Biol* **38:**267–278, 1985.

87. Leijh PC, Van Den Barselaar M, van Zwet TL, et al: Requirement of extracellular complement and immunoglobulin for intracellular killing of micro-organisms by human monocytes. *J Clin Invest* **63:**772–784, 1979.
88. Lawrence DA, Weigle WO, Spiegelberg HL: Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. *J Clin Invest* **55:**368–376, 1975.
89. Whaley K: Biosynthesis of the complement components and the regulatory proteins of the alternative complement pathway by human peripheral blood monocytes. *J Exp Med* **151:**501–516, 1980.
90. Shigeoka AO, Hall RT, Hemming VG, et al: Role of antibody and complement in opsonization of group B streptococci. *Infect Immun* **21:**34–40, 1978.
91. Wardley RC, Rouse BT, Babiuk LA: Antibody dependent cytotoxicity mediated by neutrophils: A possible mechanism of antiviral defense. *J Reticuloendothel Soc* **19:**323–332, 1976.
92. Brogen M, Sagone AL Jr: The metabolic response of human phagocytic cells to killed mumps particles. *J Reticulothel Soc* **27:**13–22, 1980.
93. Katz P, Simone CB, Henkart PA, et al: Mechanisms of antibody-dependent cellular cytotoxicity. *J Clin Invest* **65:**55–63, 1980.
94. Clark RA, Klebanoff SJ: Studies on the mechanism of antibody-dependent polymorphonuclear leukocyte-mediated cytotoxicity. *J Immunol* **119:**1413–1418, 1977.
95. Borregaard N, Kragballe K: Role of oxygen in antibody-dependent cytotoxicity mediated by monocytes and macrophages. *J Clin Invest* **66:**676–683, 1980.
96. Seim S, Espevik T: Toxic oxygen species in monocyte-mediated antibody-dependent cytotoxicity. *J Reticuloendothel Soc* **33:**417–428, 1983.
97. Hafęman DG, Lucas ZJ: Polymorphonuclear leukocyte-mediated, antibody-dependent, cellular cytotoxicity against tumor cells: Dependence on oxygen and the respiratory burst. *J Immunol* **123:**55–62, 1979.
98. Storkus WJ, Dawson JR: Oxygen-reactive metabolites are not detected at the effector–target interface during natural killing. *J Leukocyte Biol* **39:**547–557, 1986.
99. Klassen DK, Sagone AL Jr: Evidence for both oxygen and non-oxygen dependent mechanisms of antibody sensitized target cell lysis by human monocytes. *Blood* **56:**985–992, 1980.
100. Archibald AC, Cheung K, Robinson MF: The interaction of lymphocyte surface-bound immune complexes and neutrophils. *J Immunol* **131:**207–211, 1983.
101. Sacks T, Moldow CF, Craddock PR, et al: Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes an in vitro model of immune vascular damage. *J Clin Invest* **61:**1161–1167, 1978.
102. Till GO, Johnson RJ, Kunkel R, Ward PA: Intravascular activation of complement and acute lung injury. *J Clin Invest* **69:**1126–1135, 1982.
103. Ward PA, Till, Kunkel R, et al: Evidence of role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *J Clin Invest* **72:**789–801, 1983.
104. Cooke E, Hallett MB: The role of C-kinase in the physiological activation of the neutrophil oxidase. *Biochem J* **232:**323–327, 1985.
105. van der Meulen FW, van der Hart M, Fleer A, et al: The role of adherence to human mononuclear phagocytes in the destruction of red cells sensitized with non-complement binding IgG antibodies. *Br J Haematol* **38:**541–549, 1978.
106. Conkling PR, Klassen DK, Sagone AL: Comparison of antibody-dependent cytotoxicity mediated by human polymorphonuclear cells, monocytes, alveolar macrophages. *Blood* **60:**1290–1297, 1982.
107. Lee D, Hoidal J, Garlich D et al: Opsonin-independent phagocytosis of surface-adherent bacteria by human alveolar macrophages. *J Leukocyte Biol* **36:**689–701, 1984.
108. Eissenberg LG, Goldman WE: Histoplasma capsulatum fails to trigger release of superoxide from macrophages. *Infect Immun* **55:**29–34, 1987.
109. Abramson JS, Mills EL, Giebink GS, et al: Depression of monocyte and polymorphonuclear leukocyte oxidative metabolism and bactericidal capacity by influenza virus. *Infect Immun* **35:**350–355, 1982.

110. Copeland E, Rinehart J, Lewis M, et al: The mechanism of retrovirus suppression of T cell proliferation. *J Immunol* **131**:2017–2020, 1983.
111. Anderson V, Hellung-Larsen FP, Shrenson SF: Optimal oxygen tension for human lymphocytes in culture. *J Cell Physiol* **72**:149–152, 1968.
112. Anderson V, Anderson AB, Skovbjerg H, et al: Structure of mitochondria in lymphocytes culture at different oxygen tensions. *Acta Pathol Microbiol Scand [A]* **78**:537–544, 1970.
113. Mizrahi A, Vosseller GV, Yagi Y, et al: The effect of dissolved oxygen partial pressure on growth, metabolism and immunoglobulin production in a permanent human lymphocyte cell line culture. *Proc Soc Exp Biol Mod* **139**:118–122, 1972.
114. Kilburn DG, Morley M, Yensen J: The influence of dissolved oxygen on the mitogen responses of mouse lymphocytes. *J Cell Physiol* **87**:307–311, 1975.
115. Sagone AL Jr: Effect of hyperoxia on the carbohydrate metabolism of human lymphocytes. *Am J Hematol* **18**:269–274, 1985.
116. Rabinowitz Y, Lubrano T, Wilhite BA, et al: Lactic dehydrogenase of cultured lymphocytes: Response to environmental conditions. *Exp Cell Res* **48**:675–678, 1967.
117. Fridovich I: The biology of oxygen radicals. *Science* **201**:875–880, 1978.
118. Harlan JM, Levine JD, Callahan KS, et al: Glutathione Redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxide. *J Clin Invest* **73**:706–713, 1984.
119. Arrick BA, Nathan CF, Cohn ZA: Inhibition of glutathione synthesis augments lysis of murine tumor cells by sulfhydryl-reactive antineoplastics. *J Clin Invest* **71**:258–267, 1983.
120. Stankova L, Rigas DA, Keown P, et al: Leukocyte ascorbate and glutathione: Potential capacity for inactivating oxidants and free radicals. *J Reticuloendothel Soc* **21**:97–102, 1977.
121. Marklund S, Nordensson I, Back O: Normal CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione peroxidase in Werner's syndrome. *J Gerontol* **36**:405–409, 1981.
122. Meerhof LJ, Roos D: An easy, specific and sensitive assay for the determination of the catalase activity of human blood cells. *J Reticuloendothel Soc* **28**:419–425, 1980.
123. Arrick B, Nathan C: Glutathione metabolism as a determinant of therapeutic efficacy. A review. *Cancer Res.* **44**:4224–4232, 1984.
124. Roos D, Weening R, Voetman A, et al: Protection of phagocytic leukocytes by exogenous glutathione: studies in a family with glutathione deficiency reductase deficiency. *Blood* **53**:851–866, 1979.
125. Spielberg SP, Boxer LA, Oliver JM, et al: Oxidative damage to neutrophils in glutathione synthetase deficiency. *Br J Haematol* **42**:215–223, 1979.
126. Freeman BA, Crapo JD: Biology of disease-free radicals and tissue injury. *Lab Invest* **47**:412–426, 1982.
127. Chance B, Sies H, Boveris A: Hydroperoxide metabolism in mammalian organs. *Physiol Rev* **59**:527–605, 1979.
128. Baral E, Blomgen H: Response of human lymphocytes to mitogenic stimuli after irradiation in vitro. *Acta Radiol Ther Phys Biol* **15**:149–161, 1976.
129. Braeman J, Moore J: The lymphocyte response to phytohemagglutinin after in vitro radiation. *Br J Radiol* **47**:297, 1974.
130. Roberts W, Kartha M, Sagone AL Jr: Effect of irradiation on the hexose monophosphate shunt pathway of human lymphocytes. *Radiat Res* **79**:601–610, 1979.
131. Wasserman J, Petrini B, Blomgren H: Radiosensitivity of T-lymphocyte subpopulations. *J Clin Lab Immunol* **7–8**:139–140, 1982.
132. Kleinerman ES, Decker JM, Muchmore AV: In vitro cellular regulation of monocyte function: Evidence for a radiosensitive suppressor. *J Reticuloendothel Soc* **30**:373–380, 1981.
133. Stefani S, Kerman R: Lymphocyte response to phytohemagglutinin before and after radiation therapy in patients with carcinomas of the head and neck. *J Laryngol Otol* **91**:605–610, 1977.
134. Anderson RE, Standefer JC, Scaletti JV: Radiosensitivity of defined populations of lymphocytes. *Cell Immun* **33**:45–61, 1977.
135. Merz T, Hazra T, Ross M, et al: Transformation delay of lymphocytes in patients undergoing radiation therapy. *AJR* **127**:337–339, 1976.

136. Howard-Flanders P, Levin J, Theriot L: Reactions of DNA radicals with sulfhydryl in x-irradiated bacteriophage systems. *Radiat Res* **18:**593–606, 1963.
137. Goscin S, Fridovich I: Superoxide dismutase and the oxygen effect. *Radiat Res* **56:**565–569, 1973.
138. Scholes G, Weiss J: Oxygen effects and formation of peroxides in aqueous solution. *Radiat Res* **1** (suppl):177–189, 1959.
139. Oberley L, Lindgren A, Baker et al: Superoxide ion and the cause of the oxygen effect. *Radiat Res* **68:**320–328, 1976.
140. Lavelle F, Michelson M, Mitrijene: Biological protection by superoxide dismutase. *Biochem Biophys Res Commun* **55:**350–357, 1973.
141. Konings AWT, Oosterloo SK: Radiation effects on membranes. *Radiat Res* **81:**200–207, 1980.
142. Stankova L, Rigas D, Head C, et al: Determinants of resistance to radiation injury in blood granulocytes from normal donors and from patients with myeloproliferative disorders. *Radiat Res* **80:**49–60, 1979.
143. Edsmyr F, Huber W, Menander KB: Orgotein efficacy in ameliorating side effects due to radiation therapy. *Current Ther Res* **19:**198–211, 1976.
144. Westman G, Marklund SL: Diethyldithiocarbamate, a superoxide dismutase inhibitor, decreases the radioresistance of chinese hamster cells. *Radiat Res* **83:**303–311, 1980.
145. McLennan G, Oberley LW, Autor AP: The role of oxygen-derived free radicals in radiation-induced damage and death of nondividing eucaryotic cells. *Radiat Res* **84:**122–132, 1980.
146. Kiefer J: Does singlet oxygen contribute to the oxygen effect. *Int J Radiat Biol* **34:**587–588, 1978.
147. Helf KD: Interactions of radioprotectors and oxygen in cultured mammalian cells. *Radiat Res* **101:**424–433, 1985.
148. Russo A, Mitchell JB, Finkelstein E, et al: The effects of cellular glutathione elevation on the oxygen enhancement ratio. *Radiat Res* **103:**232–239, 1985.
149. Morse M, Dahl R: Cellular glutathione is a key to the oxygen effect in radiation damage. *Nature (Lond)* **271:**660–662, 1978.
150. Sagone AL Jr: Role of the hexose monophosphate shunt in cellular protection against radiation damage, in Rodgers M, Powers E (ed): *Oxygen and Oxy-radicals in Chemistry and Biology*. New York, Academic, 1981, pp 725–729.
151. O'Brien RL, Parker JW: Oxidation-Induced lymphocyte transformation. *Cell* **7:**13–20, 1976.
152. Novogrodsky A, Katchalski E: Membrane site modified on induction of the transformation of lymphocytes by periodate. *Proc Natl Acad Sci USA* **69:**3207–3210, 1972.
153. Biniaminov M, Ramot B, Novogrodsky A: Effect of macrophages on periodate-induced transformation of normal and chronic lymphatic leukaemia lymphocytes. *Clin Exp Immunol* **16:**235–242, 1974.
154. Monahan TM, Fritz RR, Abell CW: Sodium periodate stimulation of normal and chronic lymphocytic leukemia lymphocytes. *Exp Cell Res* **103:**263–269, 1976.
155. Roffman E, Wilchek M: The extent of oxidative mitogenesis does not correlate with the degree of aldehyde formation of the T lymphocyte membrane. *J Immunol* **137:**40–44, 1986.
156. Sagone AL Jr, Kamps S, Campbell R: The effect of oxidant injury on the lymphoblastic transformation of human lymphocytes. *Photochem Photobiol* **28:**909–915, 1978.
157. Lipsky PE: Immunosuppression by D-penicillamine in vitro. *J Clin Invest* **73:**53–65, 1984.
158. El-Hag A, Lipsky PE, Bennett M, et al: Immunomodulation by neutrophil myeloperoxidase and hydrogen peroxide: Differential susceptibility of human lymphocyte functions. *J Immunol* **136:**3420–3426, 1986.
159. El-Hag A, Clark RA: Down-regulation of human natural killer activity against tumors by the neutrophil myeloperoxidase system and hydrogen peroxide. *J Immunol* **133:**3291–3297, 1984.
160. Grever MR, Thompson VN, Balcerzak SP, Sagone AL Jr: The effect of oxidant stress on human lymphocyte cytotoxicity. *Blood* **56:**284–288, 1980.
161. Whisler RL, Newhouse YG: Inhibition of human B lymphocyte colony responses by endogenous synthesized hydrogen peroxide and prostaglandins. *Cell Immunol* **69:**34–45, 1982.
162. Kraut EH, Sagone AL Jr: The effect of oxidant injury on the lymphocyte membrane and functions. *J Lab Clin Med* **98:**697–703, 1981.

163. Sugar AM, Chahal RS, Brummer E, et al: The iron-hydrogen peroxide-iodide system is fungicidal: Activity against the yeast phase of blastomyces dermatitidis. *J Leukocyte Biol* **36:**545–548, 1984.
164. Clark RA, Klebanoff SJ, Einstein AB, et al: Peroxidase–H_2O_2–halide system: Cytotoxic effect on mammalian tumor cells. *Blood* **45:**161–170, 1975.
165. Weiss SJ: The role of superoxide in the destruction of erythrocyte targets by human neutrophils. *J Biol Chem* **255:**9912–9917, 1980.
166. Weiss SJ: Oxidative autoactivation of latent collangenase by human neutrophils. *Science* **227:**747–749, 1985.
167. Carp H, Janoff A: Potential mediator of inflammation phagocytic-derived oxidants suppress the elastase-inhibitory capacity of $alpha_1$-proteinase inhibitor in vitro. *J Clin Invest* **66:**987–995, 1980.
168. Farber CM, Liebes LF, Kanganis DN, et al: Human B lymphocytes show greater susceptibility to H_2O_2 toxicity than T lymphocytes. *J Immunol* **132:**2543–2546, 1984.
169. Seaman WE, Gindhart TD, Blackman MA, et al: Natural killing of tumor cells by human peripheral blood cells. *J Clin Invest* **67:**1324–1333, 1981.
170. Urbaniak SJ: ADCC (K-cell) lysis of human erythrocytes sensitized with rhesus alloantibodies. *Br J Haematol* **42:**315–328, 1979.
171. Ramos OF, Mascucci M, Klein E: Modulation of human blood lymphocyte cytotoxicity for the phorbol ester tumor promoter $P(Bu)_2$: Increase of target binding, impairment of effector recycling and activation of lytic potential which is independent of IL-2. *Cell Immunol* **91:**178–192, 1985.
172. Kay HD, Smith DL: Regulation of human lymphocyte-mediated natural killer (NK) cell activity. *J Immunol* **130:**475–483, 1983.
173. Dallegri F, Patrone F, Frumento G: Down-regulation of K cell activity by neutrophils. *Blood* **65:**571–577, 1985.
174. Nissen-Meyer J, Kildahl-Andersen O, Austgulen R: Human monocyte-released cytotoxic factor: Effect on various cellular functions, and dependency of cytolysis on various metabolic processes. *J Leukocyte Biol* **40:**121–132, 1986.
175. Niwa Y, Sakane T, Shingu, M, et al: Effect of stimulated neutrophils from the synovial fluid of patients with rheumatoid arthritis on lymphocytes—A possible role of increased oxygen radicals generated by the neutrophils. *J Clin Immunol* **3:**228–240, 1983.
176. de Vries JE, Caviles AP Jr, Bont WS, et al: The role of monocytes in human lymphocyte activation by mitogens. *J Immunol* **122:**1099–1107, 1979.
177. Lause DB: Suppressed lymphocyte reactivity following lymphocyte interaction with macrophages. *J Reticuloendothel Soc* **26:**775–786, 1979.
178. Novogrodsky A, Patya M, Rubin A, et al: Inhibition of B-adrenergic stimulation of lymphocyte adenylate cyclase by phorbol myristate acetate is mediated by activated macrophages. *Biochem Biophys Res Commun* **104:**389–393, 1982.
179. Metzger Z, Hoffeld JT, Oppenheim JJ: Macrophage-mediated suppression. *J Immunol* **124:**983–988, 1980.
180. Fisher RI, Bostick-Bruton F: Depressed T cell proliferative responses in Hodgkin's disease: Role of monocyte-mediated suppression via prostaglandins and hydrogen peroxide. *J Immunol* **129:**1770–1774, 1982.
181. Bomalaski JS, Clark MA, Douglas SD: Enhanced Phospholipase A_2 and C activities of peripheral blood polymorphonuclear leukocytes from patients with rheumatoid arthritis. *J Leukocyte Biol* **38:**649–654, 1985.
182. Randazzo B, Hirschberg T, Hirschberg H: Cytotoxic effects of activated human monocytes and lymphocytes to anti-D-treated human erythrocytes in vitro. *Scand J Immunol* **9:**351–358, 1979.
183. Nyholm RE, Currie GA: Monocytes and macrophages in malignant melanoma in Lysis of antibody-coated human erythrocytes as an assay of monocyte function. *Br J Cancer* **37:**337–344, 1978.
184. Bass DA, Olbrantz P, Szejda P, et al: Subpopulations of neutrophils with increased oxidative product formation in blood of patients with infection. *J Immunol* **136:**860–866, 1984.
185. Bass DA, Grover WH, Lewis JC, et al: Comparison of human eosinophils from normals and patients with eosinophilia. *J Clin Invest* **66:**1265–1273, 1980.

186. Kitahara M, Eyre HJ, Hill HR: Monocyte functional and metabolic activity in malignant and inflammatory diseases. *J Lab Clin Med* **93:**472–479, 1979.
187. Deshazo RD, Ewel C, Londono S, et al: Evidence for the involvement of monocyte-derived toxic oxygen metabolites in the lymphocyte dysfunction of Hodgkin's disease. *Clin Exp Immunol* **46:**313–320, 1981.
188. Nagai H, Fisher RI, Cossman J, et al: Decreased expression of Class II major histocompatibility antigens on monocytes from patients with Hodgkin's disease. *J Leukocyte Biol* **39:**313–321, 1986.
189. Metzger Z, Hoffeld JT, Oppenheim JJ: Regulation by PGE_2 of the production of oxygen intermediates by LPS-activated macrophages. *J Immunol* **127:**1109–1113, 1981.
190. Bray RA, Brahmi Z: Role of lipoxygenation in human natural killer cell activation. *J Immunol* **136:**1783–1798, 1986.
191. Singh D, Greenwald J, Bianchine J, et al: Evidence for the generation of hydroxyj radical during arachidonic acid metabolism by human platelets. *Am J Hematol* **11:**233–240, 1981.
192. Harrel RA, Cianciolo CJ, Copeland TD: Suppression of the respiratory burst of human monocytes by a synthetic peptide homologous to envelope proteins of human and animal retroviruses. *J Immunol* **186:**3517–3528, 1986.
193. Cianciolo GJ, Copeland TD, Oroszlan S, et al: Inhibition of lymphocyte proliferation by a synthetic peptide homologous to retroviral envelope proteins. *Science* **230:**453–455, 1985.
194. Rhodes J, Bishop M, Benfield J: Tumor Surveillance: How tumors may resist macrophage-mediated host defense. *Science* **203:**179–182, 1979.