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Chemiluminescence Response of Human Leukocytes: Influence of Medium Components on Light Production

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Light-producing reactions have been reported to occur after phagocytosis of opsonized particles by human polymorphonuclear neutrophils, eosinophils, and monocytes. Such chemiluminescence appears to be related to the generation of singlet oxygen, superoxide, and hydroxyl radicals, which have also been implicated as microbicidal agents. In examining the influences of various medium components on leukocyte chemiluminescence, we have observed that the amount of light measured is increased by addition of soluble protein, the amino acids tyrosine and tryptophane, or excess zymosan to the reaction medium. These agents appear to produce their effect not by increasing the rate of phagocytosis, but by providing substrate for secondary light-producing reactions. Polystyrene particles do not provide a suitable substrate for such secondary light-producing reactions. This is evidenced by the failure of ingested latex to stimulate high levels of chemiluminescence in the cellular system and their failure to augment light production in two noncellular chemiluminescent reactions. Some of the light generated in the cellular chemiluminescence response may derive from secondary reactions, which occur outside of the phagocyte. Support for this phenomenon is provided by two experiments. In one, addition of supplementary, nonopsonized zymosan to the reaction, after phagocytosis of opsonized zymosan is complete, resulted in an increased level of chemiluminescence. In another, addition of nonopsonized zymosan, together with latex particles, resulted in a significant increase in chemiluminescence. The results of the latter experiment also support our hypothesis that latex does not provide an appropriate substrate for secondary light-producing reactions. These observations suggest that leukocytes activated by phagocytosis generate electronically activated radicals which act intra- and extracellularly and that the amino acids tyrosine and tryptophane may provide one substrate through which these agents act.

Light-producing reactions have been reported to occur after ingestion of bacteria, yeast, or zymosan particles by human polymorphonuclear neutrophils (2), eosinophils (11), and monocytes (10, 12). The apparent relationship of this chemiluminescence to the generation of intermediate products of oxygen reduction (2, 4), together with the microbicidal potential of these molecular species (7, 8), has spurred increasing interest in this phenomenon. Recent reports have, for example, attempted to characterize the spectrum of the emitted light (4, 14) and demonstrated a possible contribution of the particle itself as a substrate for light-producing reactions (4, 11).

In selecting the conditions we currently use for routine chemiluminescence studies, we have observed that several components of the reaction medium can have profound influence on the amount of light measured. Addition of zymosan in excess of that required to achieve a maximal rate of phagocytosis or addition of certain amino acids or soluble protein increases the chemiluminescence response. Such observations may provide a partial explanation for differences in magnitudes of chemiluminescence reported from different laboratories. With other experiments we have demonstrated that excess zymosan, tyrosine, and soluble protein may affect chemiluminescence by providing substrate for extracellular, secondary light-producing reactions. These results support the suggestion that a major portion of the light measured in cellular chemiluminescence reactions may be the product of such secondary reactions in which the particle itself provides the necessary substrate (4, 11).

MATERIALS AND METHODS

Preparation of cells. Blood was drawn from healthy adult volunteers in sterile, disposable syringes containing 10 U of sodium heparin (The Upjohn Co.,

Kalamazoo, Mich.) per ml of blood collected. The blood was centrifuged at 200 × *g* for 15 min, after which the plasma was drawn off and the buffy coat leukocytes, together with the upper 0.5 cm of erythrocytes, were resuspended in the plasma. Contaminating erythrocytes were eliminated by gravity sedimentation at 37°C, and the unpurified leukocytes were recovered from the leukocyte-rich plasma by centrifugation at 200 × *g* for 15 min. The leukocytes were then resuspended in tissue culture medium (minimum essential medium [MEM] supplemented with 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml; all from Grand Island Biological Co., Grand Island, N.Y.) at a concentration no greater than 2×10^7/ml, and 8- to 10-ml volumes of this cell suspension were layered over 3 ml of lymphocyte separation medium (Bionetics Laboratory Products, Kensington, Md.) in 15-ml tubes. After centrifugation at 400 × *g* for 40 min, polymorphonuclear neutrophils (PMN) were recovered from the sediment and contaminating erythrocytes were removed by hypotonic lysis (9). The cells were then washed three times in MEM before culture and resuspended in phenol red-free Hanks balanced salt solution (HBSS), unless otherwise specified, at a concentration of 10^6 phagocytes per ml.

Slides of all isolated cell populations were prepared with the aid of a Cytospin centrifuge (Shandon Southern Instruments, Sewickley, Pa.) and stained with Wright stain, and contamination was monitored microscopically. Purified populations of PMN consistently contained fewer than 2% cells of other types.

Preparation of opsonized zymosan. Zymosan particles (Schwarz/Mann, Orangeburg, N.Y.) were opsonized with fresh autologous serum by the method of Wardlaw and Pillemer (16), except that the dry zymosan was wetted in phosphate-buffered saline before the opsonization step. The opsonized particles were recovered by centrifugation at 1,800 × *g* for 5 min, the supernatant was discarded, and the particles were resuspended in HBSS at a final concentration of 10 mg/ml.

Quantitation of chemiluminescence. Chemiluminescence was measured in a liquid scintillation spectrometer (model LS-330, Beckman Instruments, Irvine, Calif.) at ambient temperature and adjusted as described by Stanley and Williams (13) and Stjernholm et al. (15). Glass counting vials (no. 161698, Beckman Instruments), which had been dark adapted, were transferred to the spectrometer and filled under red illumination. Initially, 10 counting vials placed at 30-space intervals were counted empty for 0.1 min/vial on two to three counting cycles to monitor luminescence for each vial. Vials with excessively high counts were replaced in order to match background counts among the vials as closely as possible. Subsequently, 2×10^6 PMN suspended in HBSS were added to each test vial in a 2-ml volume, and the vials were counted again to obtain background values. These values were never significantly greater than those obtained for the empty vials (8,000 to 15,000 cpm). Finally, a 0.5-ml volume of opsonized zymosan (5 mg) was added to the first vial in the sequence, the vial was swirled, and, after a 37-s delay, the counting cycle was begun. As the first vial entered the counting chamber, zymosan was added to the next vial, and so on, until all vials had been filled. Each vial was then counted for a 0.1-min interval in each 7-min cycle for the duration of the assay.

Presentation of data. For a majority of experiments, we have chosen to present our data in terms of net peak counts-per-minute values. This choice has been prompted by our observation that counts-per-minute values may remain above background for more than 8 h after initiation of the assay. Use of the peak value, which can be obtained within 30 min, thereby considerably shortens the time requirement and also minimizes the influences of substrate exhaustion, pH changes, and settling out of cells and particles. The rate of particle uptake by PMN has been observed to be extremely rapid under the conditions described, such that phagocytosis is essentially complete within 1 min after initiation of the reaction. Therefore, minor differences in phagocytic rates can also have minimal influence on the peak values for chemiluminescence.

RESULTS

Influence of medium components. The medium we used for our earlier studies was supplemented to contain 1 g of gelatin per 100 ml. Data presented in Fig. 1 demonstrate that addition of this soluble protein to the reaction medium significantly enhances the chemiluminescence response. Supplementation of the medium with gelatin at a level of 1 g/100 ml was observed to increase the peak counts per minute by more than 90%. Raising the level of gelatin to 3 g/100 ml augmented light production maximally, increasing the peak counts per minute by

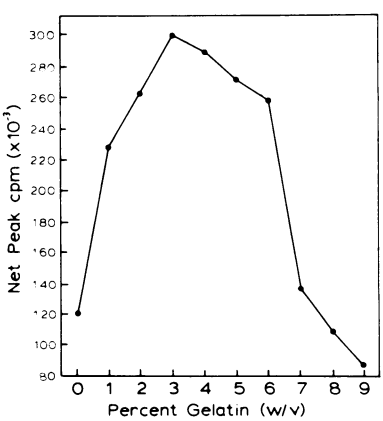

FIG. 1. *Effect of addition of soluble protein to reaction medium on the chemiluminescence response of human PMN. The chemiluminescence response of PMN induced by phagocytosis of opsonized zymosan was compared by using indicator-free HBSS without gelatin or supplemented to contain 1 to 9 g of gelatin per 100 ml. Addition of gelatin to the leukocytes, in the absence of opsonized zymosan, did not produce a chemiluminescent response.*

150%. Addition of greater amounts of gelatin resulted in a progressive decrease in peak chemiluminescence, possibly due to the resulting increase in medium density.

Phenol red indicator has been deleted from our reaction medium due to its negative effect on chemiluminescence. Data presented in Fig. 2 demonstrate that 35% more light, measured as peak counts per minute, is obtained with indicator-free HBSS (2nd bar) than with HBSS containing 0.01 mg of phenol red per ml (1st bar). This effect may be due to absorption of some of the light produced. Since various media with indicator contain from 0.005 (RPMI 1640) to 0.02 (medium 199) mg of phenol red per ml, the influence of this medium component must be considered in chemiluminescence assays.

In comparing chemiluminescence responses of leukocytes suspended in various reaction media, we have observed that greater responses are obtained when more complete media are used. For example, MEM is supplemented with essential amino acids and the vitamins calcium-pantothenate, choline chloride, folic acid, nicotinamide, pyridoxal HCl, riboflavin, and thiamine HCl. To determine whether either or both of these supplements is responsible for the greater chemiluminescence responses obtained with MEM, we added vitamin and essential amino acid solutions (no. 112 and 113G, respectively, Grand Island Biological Co.) separately to HBSS. Data presented in Fig. 2 demonstrate that addition of vitamin solution had no effect on the chemiluminescence response (cf. 1st and 3rd bars), but addition of essential amino acid solution increased the chemiluminescence response (cf. 1st and 4th bars) to a level approximating that obtained with MEM as the reaction medium (5th bar).

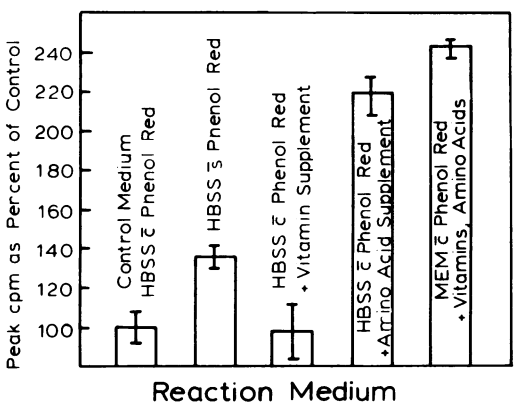

FIG. 2. *Influence of phenol red, vitamin, and amino acid supplements on the chemiluminescence response of human PMN induced by phagocytosis of opsonized zymosan. HBSS and MEM with indicator contained phenol red at a concentration of 0.01 mg/ml. Supplementation of HBSS with vitamins and essential amino acids was done by addition of the respective concentrated mixtures, obtained commercially.*

Influence of individual amino acids. Subsequent experiments were conducted to identify the amino acid(s) responsible for augmentation of chemiluminescence. In these experiments, 21 amino acids were added individually to HBSS containing phenol red at concentrations cited for the respective species (5). The amino acids tested included the L-forms of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophane, tyrosine, and valine (kit no. LA-21, Sigma Chemical Co., St. Louis, Mo.). Of these, the only amino acids observed to influence significantly the amount of chemiluminescence measured were tyrosine, tryptophane, and cysteine (data not shown).

To determine the dose-related influences of tyrosine, tryptophane, and cysteine, these amino acids were added to indicator-free HBSS at concentrations over the range of 0.02 to 0.56 mM. The effects of all three amino acids were dose dependent (Fig. 3). Addition of tyrosine increased the chemiluminescence response of PMN by approximately 15% at a concentration of 0.04 mM and by more than 75% at a concentration of 0.56 mM. Addition of tryptophane also increased PMN chemiluminescence. Like tyrosine, at a concentration of 0.04 mM, the amount of light measured was increased by approximately 15%. Unlike tyrosine, the maximal increase in chemiluminescence observed with tryptophane was approximately 35%. Addition of cysteine, unlike tyrosine and tryptophane, was found to decrease the PMN chemiluminescence response. Cysteine at a concentration of 0.05 mM decreased the amount of light measured by approximately 25%. Maximal inhibition of approximately 60% was obtained with cysteine at a concentration $\geq$0.25 mM. Since cysteine is not one of the amino acids present in MEM as obtained from Grand Island Biological Co., the positive effects of tyrosine and tryptophane alone must account for the greater chemiluminescence response measured using MEM as the reaction medium. We have not assessed whether the influence of these amino acids is affected by the presence of phenol red.

To determine whether the enhancing effect of tyrosine and tryptophane is specific for the cellular chemiluminescence response, we also assessed the effect of tyrosine on two noncellular light-producing systems. Data presented in Fig.

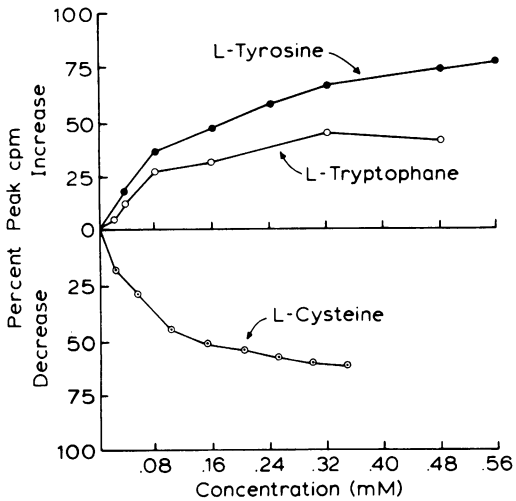

FIG. 3. *Dose response of the L-amino acids tyrosine, tryptophane, and cysteine on the chemiluminescence response of human PMN induced by phagocytosis of opsonized zymosan. Addition of tyrosine or tryptophane to the leukocytes, in the absence of opsonized zymosan, did not produce a chemiluminescence response.*

4 demonstrate that the addition of 10 μM tyrosine to vials containing 22 mM H_2O_2 and 1 mM NaClO or 22 mM H_2O_2 and 0.025 mg of horseradish peroxidase per ml in 2 ml of HBSS similarly enhances the chemiluminescence measured from these noncellular systems. No light was measured from vials containing H_2O_2 and tyrosine alone.

Influence of zymosan concentration. As expected, zymosan concentration also influences the leukocyte chemiluminescence response. Unexpected, however, was the observation that addition of zymosan in excess of that required to achieve a maximal rate of phagocytosis significantly increases the amount of light measured. Data illustrating this phenomenon are presented in Fig. 5. With a zymosan concentration of 0.75 mg/ml, the net peak chemiluminescence observed was approximately 80,000 cpm. This value was increased to 160,000 cpm on increasing the concentration of zymosan to 8 mg/ml. This observation emphasizes the requirement for control of particle-to-cell ratios in measurement of the leukocyte chemiluminescence response and also suggests that extracellular zymosan may influence the magnitude of chemiluminescence.

In a chemiluminescence reaction mixture containing 2×10^6 PMN and 0.75 mg of zymosan per ml, the particle-to-cell ratio is approximately 30:1. At this particle-to-cell ratio and at room temperature, phagocytosis is very rapid. Observation by light microscopy of PMN removed from such cultures at various times after addition of zymosan reveals that virtually all cells have ingested five or more particles within 1 min after initiation of the reaction. This suggests that addition of larger amounts of zymosan may be influencing the chemiluminescence response by a mechanism not involving altered rate of phagocytosis.

To establish that extracellular zymosan can contribute to the amount of light measured, we have added supplementary nonopsonized zymosan to the chemiluminescence assay. Nonopsonized zymosan has been used since it neither is phagocytized nor stimulates a chemiluminescence response under the conditions of the as-

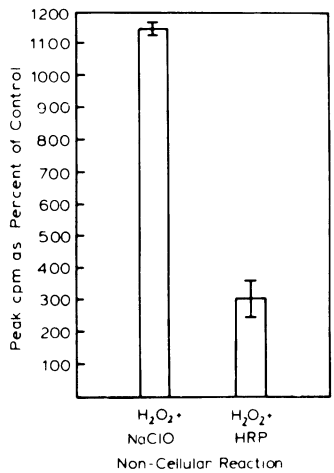

FIG. 4. *Effect of L-tyrosine on two noncellular chemiluminescence reactions, NaClO + H_2O_2 and horseradish peroxidase (HRP) + H_2O_2. These reactions were carried out in HBSS. The data are presented as mean percent increase in peak counts per minute ± standard error of the mean.*

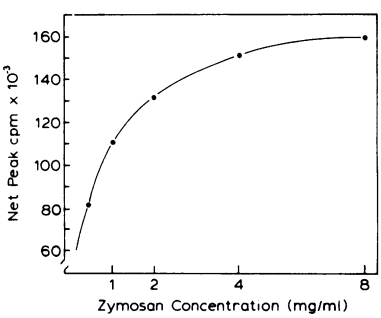

FIG. 5. *Effect of zymosan concentration on the chemiluminescence response of human PMN. With 2 × 10^6 PMN/vial, a zymosan concentration of 1 mg/ml provides a particle-cell ratio of 40:1.*

say. For this experiment we set up duplicate counting vials, each containing 2×10^6 PMN and 5 mg of opsonized zymosan in a 2.5-ml volume. After monitoring chemiluminescence for 14 min (the time at which the peak response occurs), 5 mg of nonopsonized zymosan in 0.5 ml of HBSS or an equal volume of HBSS alone was added to the respective test and control vials, and chemiluminescence was monitored for the duration the assay. Results of a typical experiment are illustrated by data presented in Fig. 6. Addition of nonopsonized zymosan increased the peak chemiluminescence response from 65,000 to 120,000 cpm in the subsequent time interval. Furthermore, the difference in light measured from the two vials remained constant from 35 min to the time of termination of the assay. Therefore, addition of zymosan in a form that is not phagocytized to an ongoing chemiluminescence response significantly increases the amount of light measured.

Nonphagocytized zymosan can also be demonstrated to augment chemiluminescence when added, with latex particles, to PMN. In our experience, ingestion of latex particles by PMN produces at most only a very low level of chemiluminescence. Since phagocytosis of latex is known to stimulate superoxide generation by neutrophils (17) and since secondary light-producing reactions may contribute significantly to the cellular chemiluminescence response, we

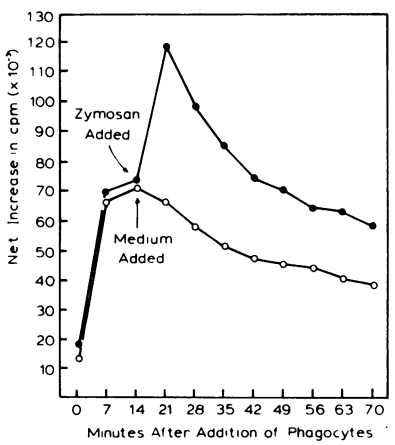

FIG. 6. *Effect of addition of nonopsonized zymosan on the chemiluminescence response of human PMN. Duplicate vials were set up to contain PMN and opsonized zymosan, and chemiluminescence was monitored for 14 min. At that time supplementary, nonopsonized zymosan in HBSS or an equivalent volume of HBSS alone was added to the respective vials, and chemiluminescence was monitored for an additional 60 min. Nonopsonized zymosan is not phagocytized and does not elicit a PMN chemiluminescence response.*

have suspected that the latex-induced chemiluminescence response is low because the latex does not provide an appropriate substrate for such reactions. To test the substrate role of latex in chemiluminescent reactions, we have examined the effect of addition of latex particles to two noncellular light-producing reactions. Nonopsonized zymosan, which has been shown to augment noncellular chemiluminescent reactions (4, 11), was used as the positive control particle. Data presented in Fig. 7 demonstrate that the addition of 1.0 mg of latex particles (polystyrene, 5.7-μm diameter; Dow Chemical Co., Indianapolis, Ind.) to vials containing 22 mM H_2O_2 and 0.76 mM NaClO or 22 mM H_2O_2 and 0.025 mg of horseradish peroxidase per ml in 2 ml of HBSS does not influence either of these light-producing reactions. Addition of an equivalent amount of nonopsonized zymosan, however, increased the light generated by these reactants by approximately 600 and 50%, respectively. Latex particles, therefore, do not appear to provide appropriate substrate for secondary light-producing reactions in either the cellular or two noncellular chemiluminescent reactions.

Since zymosan, but not latex, may provide substrate for secondary chemiluminescent reactions, we have examined the influence of addition of nonopsonized zymosan on the PMN chemiluminescence response induced by ingestion of latex particles. If latex is able to stimulate production of reactants with light-producing potential, but which cannot react with the latex as substrate, then addition of non-phagocytizable zymosan should augment the latex-induced chemiluminescence response by providing the missing substrate. To test this hypothesis, we added to replicate vials containing 2×10^6 PMN either 0.2 mg of latex (polystyrene, 5.7-μm diameter), 10 mg of nonopsonized zymosan, or latex and zymosan together in a total reaction volume of 2.5 ml. The results of a typical experiment are illustrated by data presented in Fig. 8. Addition of latex particles alone produced a constant chemiluminescence response of 5×10^3 cpm at each time interval over the 35-min assay period. Addition of nonopsonized zymosan alone produced a chemiluminescence response that varied between 10^4 and 1.5×10^4 cpm at each time interval of the assay. This response is at least 10-fold less than that which one would observe on addition of this amount of zymosan in the opsonized form (see Fig. 5). Addition of the two particles together, however, resulted in a similarly constant chemiluminescence response of approximately 3×10^4 cpm at each of the time intervals monitored. This nonadditive effect of addition of nonopsonized zy-

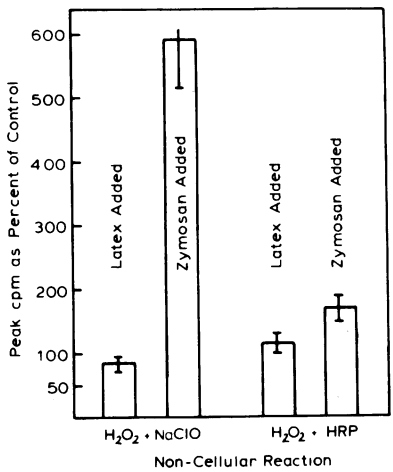

FIG. 7. *Effect of latex (polystyrene, 5.7-μm diameter) and zymosan particles on two noncellular chemiluminescent reactions, NaClO + H_2O_2 and horseradish peroxidase (HRP) + H_2O_2. These reactions were carried out in HBSS. The data are presented as mean percent increase in peak counts per minute ± standard error of the mean.*

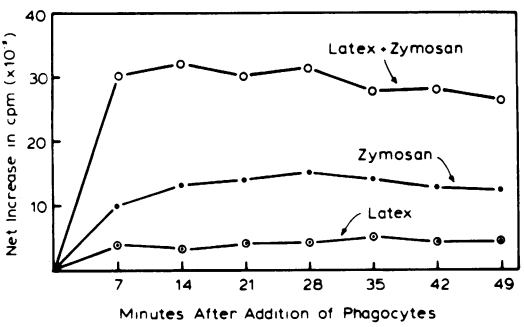

FIG. 8. *Effect of latex (polystyrene, 5.7-μm diameter) particles and nonopsonized zymosan on the PMN chemiluminescence response. Replicate vials containing 2 × 10^6 PMN received 0.2 mg of latex alone, 10 mg of nonopsonized zymosan alone, or latex and nonopsonized zymosan together.*

mosan and latex together supports our hypothesis concerning the relative substrate potentials for these particles in secondary chemiluminescent reactions. Exclusive ingestion of the latex particles in the presence of nonopsonized zymosan was verified by microscopic examination of the PMN after termination of the assay.

DISCUSSION

The influence of medium components and extracellular particles on the magnitude of the leukocyte chemiluminescence response has significance for several aspects of the chemiluminescence assay. These studies demonstrate a need for consideration of the reaction medium and particle-to-phagocyte ratio used in comparing results obtained in different laboratories. They demonstrate that a portion of the chemiluminescence measured is the result of reactions that occur outside of the phagocyte and thereby contribute to an understanding of the source of light in this assay. They also suggest that certain agents may be added to the reaction medium to improve detection of chemiluminescence and thereby permit use of smaller cell numbers or lower particle-to-phagocyte ratios.

A review of the methodologies that have been described for measurement of leukocyte chemiluminescence reveals use of a wide variety of reaction media to support this reaction. Allen et al. (2) have used RPMI 1640; Johnston et al. (6), Krebs Ringer buffer containing bovine serum albumin and dextrose; Nelson et al. (10), HBSS containing phenol red and 1% gelatin; Rosen and Klebanoff (11), modified HBSS; and Sagone et al. (12), Earle balanced salt solution supplemented with vitamins and amino acids. Similarly, the amounts of zymosan added vary from 1 (11) to 5 mg/ml (6). Since we have demonstrated that phenol red, certain amino acids, soluble protein, and excess zymosan can influence the chemiluminescence response, a direct comparison of results reported by different laboratories is not possible. We suggest, therefore, that in future studies of the leukocyte chemiluminescence response either a nonsupplemented balanced salt solution be used or medium supplementation be defined and carefully controlled. Such media should also be free of phenol red, which may absorb a portion of the light produced, and agents such as cysteine, which have reducing potential.

The source(s) of light in the leukocyte chemiluminescence response has not been identified. Allen et al. (3) have proposed that a portion of this light is the result of reactions of singlet oxygen with molecular species containing double bonds to produce excited intermediates, which provide secondary sources of light. The increase in chemiluminescence observed by Rosen and Klebanoff (11) and Cheson et al. (4) on addition of zymosan to two noncellular light-producing reactions suggests that zymosan may provide the substrate for such secondary reactions. Our observation that the amount of zymosan present in the cellular chemiluminescence reaction influences the magnitude of the response demonstrates that zymosan and microbial particles serve a similar role in the cellular reaction. The different levels of chemiluminescence we have observed using bacterial and yeast particles (10) may therefore reflect, in

part, different abilities of these particles to serve as substrate for secondary light-producing reactions. Furthermore, our observation that only very low levels of chemiluminescence are generated with latex beads as the phagocytizable particle may also be in part attributable to the failure of polystyrene to provide an appropriate substrate for such reactions. The importance of the substrate property of the ingested particle is supported by our observations that latex beads stimulate only low levels of chemiluminescence in the cellular light-producing reaction, that latex beads fail to augment two noncellular light-producing reactions, and that nonphagocytized zymosan significantly increases the amount of light produced by PMN after ingestion of latex particles.

The nature of the substrate provided by the particle in chemiluminescence reactions remains unknown. Our observation that addition of the amino acids tyrosine and tryptophane to the reaction medium increases the amount of light measured suggests that such residues in proteins of zymosan or other microbial particles may provide some of the substrate for secondary chemiluminescence reactions. This possibility is also supported by our demonstration that addition of gelatin (which contains 1 g of tyrosine per 100 g of protein) to the reaction medium results in increased chemiluminescence. Addition of 1 g of gelatin per 100 ml, which increased the chemiluminescence response by approximately 90%, is equivalent to supplementation of the reaction medium with tyrosine at a level of 0.55 mM. This level of augmentation approaches what one would expect for addition of that amount of free tyrosine, by extrapolation from the data presented in Fig. 3. The effect of addition of gelatin to the reaction medium is therefore nearly totally attributable to its tyrosine content.

It could be argued that addition of amino acids or soluble protein might alternatively influence the leukocyte chemiluminescence response by affecting the rate of phagocytosis. We believe that this is unlikely since direct assessment of phagocytosis reveals that all cells appear to be saturated with ingested zymosan in less than 1 min after initiation of the reaction. Furthermore, the effects others (4, 11) have observed on addition of zymosan to noncellular chemiluminescence reactions and those we have demonstrated for addition of tyrosine to two noncellular chemiluminescence reactions support the possibility that amino acids, soluble protein, or excess zymosan increase the chemiluminescence response at the chemical rather than the cellular level. Whether amino acids might also augment chemiluminescence by interaction with amino acid oxidases, to produce additional superoxide, is not known.

The relationship of zymosan concentration to the value for peak chemiluminescence, under conditions at which phagocytosis must be at or near its maximal rate, suggested to us that extracellular zymosan may also participate in the cellular chemiluminescence reaction. Our experiments in which nonopsonized zymosan, which neither is phagocytized nor stimulates a cellular chemiluminescence response, augments chemiluminescence support this possibility. Addition of nonopsonized zymosan at a point in time at which phagocytosis of opsonized zymosan is complete and at which peak light production has occurred produced a significant increase in the amount of light measured. Similarly, addition of nonopsonized zymosan, with latex beads, to the reaction medium produced a significant increase in the latex-induced chemiluminescence response. These observations also demonstrate the importance of secondary light-producing reactions in cellular chemiluminescence reactions.

These observations have further potential significance for microbicidal reactions occurring in vivo. If leukocytes can release activated forms of oxygen that can interact with appropriate extracellular substrate(s), if the substrate (i.e., amino acids) is altered as a consequence of this interaction, and if critical proteins containing the altered amino acid(s) are rendered inactive, then such extracellular reactions might have a microbicidal effect. This phenomenon would permit killing of nonphagocytized microbes and thereby also increase the microbicidal potential of host leukocytes.

Finally, it is always a goal in development of assays for measurement of leukocyte functions to reduce the number of cells required. The increased chemiluminescence observed on supplementation of the reaction medium with tyrosine, soluble protein, or excess nonopsonized particles may provide, like addition of luminol (1), a method for measurement of chemiluminescence with reduced cell numbers. These methods of increasing light production may have an advantage over luminol in that, unlike luminol, they do not interact with H_2O_2 to produce light. Their use for this purpose, however, will first require a clearer understanding of their role in augmentation of the cellular chemiluminescence response.

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