Title
EXPOSURE OF MICE TO OZONE: IMMUNOLOGIC AND HEMATOLOGIC EFFECTS

Author
Goodman, J.W.

Publication Date
1984-05-01

LBL-17862
Preprint

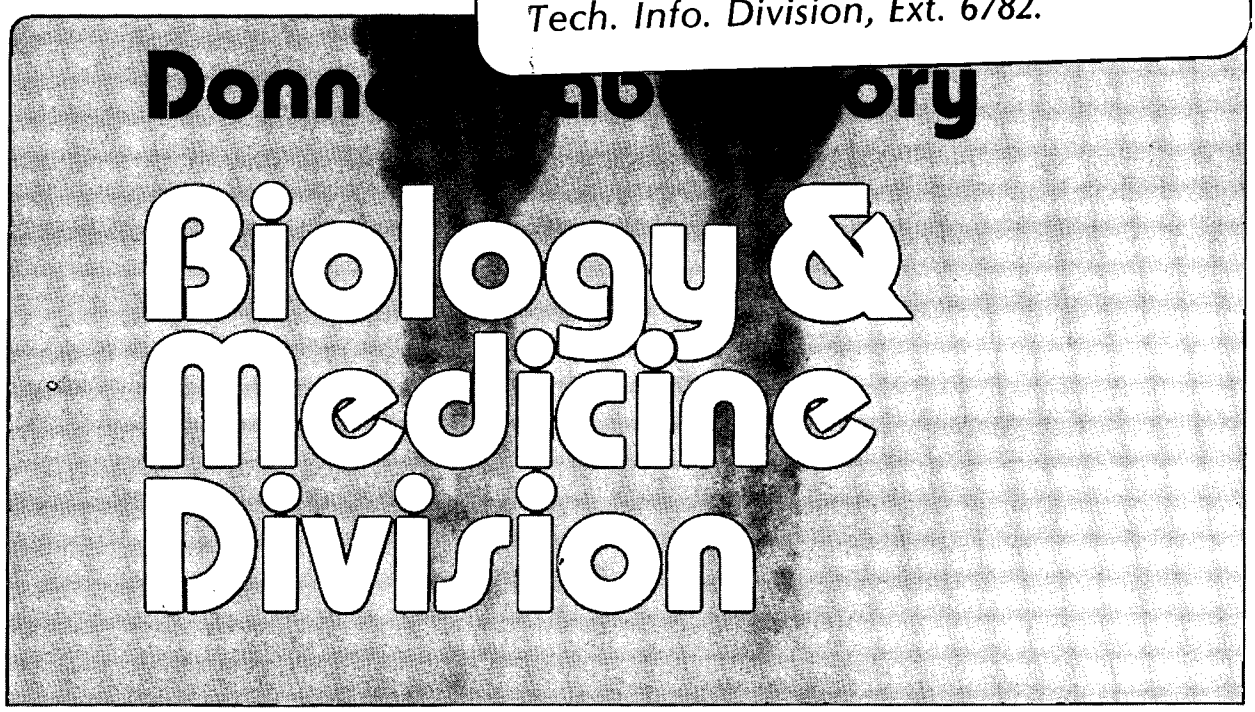
Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

Submitted to Toxicology and Applied Pharmacology

EXPOSURE OF MICE TO OZONE: IMMUNOLOGIC AND HEMATOLOGIC EFFECTS

J.W. Goodman, F.E. Peter-Fizaine, S.G. Shinpock, E.A. Hall, and D.J. Fahmie

May 1984



Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

DISCLAIMER

LBL-17862

EXPOSURE OF MICE TO OZONE:

IMMUNOLOGIC AND HEMATOLOGIC EFFECTS

Joan Wright Goodman, Françoise E. Peter-Frizaine*, Sarah Garner Shinpock,
Elizabeth A. Hall, and Darlene J. Fahmie**

Division of Biology and Medicine
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

May 1984

* Laboratory guest investigator

** Undergraduate research participant, summer, 1980.

This work was supported by the Office of Health and Environmental Research, Office of Energy Research, U.S. Department of Energy, under Contract Number DE-AC03-76SF00098.

SUMMARY

Immunologic and hematologic effects of continuous, chronic exposure of mice to ozone are reported. A consistent decrease was found in ability of spleen cells from exposed mice to engage in primary antibody formation. Results from an investigation of the possible presence of suppressor cells were inconclusive. Separation of spleen cells into adherent and nonadherent populations revealed important differences between normal and ozone-exposed mice.. Among myelopoietic progentiors examined, CFU-S, CFU-GM, CFU-E, CFU-ME, and BFU-E, only the last two named were found to be changed significantly after ozone exposure. The possible bases and implications of these findings are discussed.

Key Words: Ozone, hemopoiesis, immune response

Running head: Immunologic and hematologic effects of ozone

INTRODUCTION

It has long been known that the lung, very logically, is the site of primary injury inflicted by ozone and other harmful, gaseous environmental pollutants [1, 2]. Damage to lung tissue, which can harm the organism in various ways, definitely lowers or removes natural barriers to airborne infections with serious consequences to health [3, 4]. Although there have been studies of lung macrophage concentration and function [5], to our knowledge not much attention has been paid to the rest of the ozone-exposed animal's immune system, some elements of which are of fundamental importance in dealing with bacterial invasions. Being interested in thymus-marrow interactions and more broadly all immuno- and myelo-progenitors, we have investigated effects of ozone exposure on such cellular systems.

With the approach of first exaggerating the treatment in order to reveal major effects and later to examine them in detail, we picked the highest ozone concentration considered to be within the possible range of human exposure, one part per million [6], and used exposure durations in excess of any anticipated for the general population. In this paper we report that the primary immune response is significantly suppressed in exposed mice and that this suppression was not alleviated by removal of Lyt-2.2 bearing cells. Although there was no consistent change in several hemopoietic precursors (stem cells), assayed either in vivo or in vitro, the early burst-forming erythroid progenitor (BFU-E) was found to be increased during the first two weeks of exposure.

MATERIALS AND METHOD

Mice: (C57BL/6 ♀ X DBA/2 ♂)F_1 male hybrids, 11-14 weeks old, were used for all studies. They were purchased from the University of California production facility at 6-10 weeks of age and maintained thereafter, at least two weeks before initiation of an experiment, in conventional animal stock rooms at Lawrence Berkeley Laboratory. Mice did not vary in age by more than two weeks in any given experiment, and different ages were equally represented in all test groups.

Irradiation: Those mice to be used as recipients in spleen colony-forming unit (CFU-S) assays were exposed to 12.5Gy Cobalt irradiation at a rate of 0.22Gy/min 18-22 hours before cell transfer.

Ozone Exposure: The experimental group (O_3) was continuously exposed to 1 ppm ozone in large stainless steel environmental chambers obtained from Young and Bertke, Cincinnati, OH. Constructed with two large glass doors, the chambers admitted light on a 12 hours on/12 hours off schedule. An OREC ozone generator was used with oxygen as a gas source, and chamber ozone content was monitored daily by means of a spectrophotometric analyzer (Dasibi Model 1003-AH). Within a chamber mice were housed in stainless steel wire cages constructed so that there was a free flow of O_3. No bedding was used, but chambers were cleaned three times a week at which times unused food and water were replaced with fresh supplies. Five to 10 mice were put in each cage, and the number of animals per chamber was kept constant for the duration of a given experiment.

Control Groups: One group of control mice (Sham) was kept under identical conditions, including air flow, in the same kind of chamber as was used for O_3 exposure but without the addition of O_3. A second control group (N) was removed from the stock room along with mice to be placed in chambers but was housed conventionally, 5 per cage, in a separate, smaller room designated for experimental animals.

Stem Cell Assays: The standard CFU-S method of Till and McCulloch [7] was carried out in irradiated isogenic mice. Spleens were removed 8 days after transplantation and were fixed in Tellyesniczki's solution. CFU-E were cultured with relatively high specific activity erythropoietin (approximately 1140 units per mg protein, obtained through a distribution program from the National Heart, Lung, and Blood Institute) at 0.25 units/ml, according to the method of Urabe and Murphy [8]. The assay for more primitive BFU-E followed the method of McLeod et al. [9] and used crude (4.2 units/mg) human urinary erythropoietin (EP), kindly supplied by the late Joseph F. Garcia, at 3 units/ml. The method of McLeod et al. [10] was slightly modified (i.e., we used 7-day conditioned medium instead of 4-day) to analyze CFU-ME, and that of Stewart [11] for CFU-GM. Mouse endotoxin serum, at a final concentration of 1-1/2 to 2-1/2% (depending on results from titration of the particular lot of serum) was used as the source of colony stimulating factor (CSF). Colonies were enumerated on day 7.

Plaque-forming cells (PFC): The hemolytic plaque assay methods described by Mishell and Shiigi [12] for primary and secondary in vitro immune response to sheep erythrocytes (SRBC) were used without modification.

Spleen and marrow cell suspensions: Spleens were disrupted in medium RPMI 1640 with the use of a teflon pestle loosely fitted in a heavy-wall, round-bottom tube. Tibias were flushed through (i.e. into, then out of a syringe) a 24 gauge needle and then flushed twice through a 27 gauge needle in phosphate buffered saline (PBS), pH 7.2-7.4. A hemocytometer count was used as the basis for adjusting the concentration of eosin-excluding cells appropriately for any given procedure. Sterile precautions were taken in all instances in which cells were to be cultured in vitro.

Adherent (Ad) Cells: Spleen cells, after ammonium chloride/Tris (ACT) treatment [12] were suspended at 10^7 cells/ml in medium RPMI 1640 containing 10% fetal bovine serum (FBS). FBS, 10% for Ad, 5% for Nad, was included in medium throughout separation procedures. Five ml portions of the cell suspension were allowed to stand in 100 mm plastic petri dishes (Falcon) at 37°C for two hours after which nonadherent cells were gently decanted. Adherent cells were carefully rinsed three times with warm (37°C) medium. Decanted cells were pooled and counted for estimates of recovery, but they were not used as the nonadherent (Nad) fraction in subsequent experiments (vide infra). Ad cells were then washed with cold medium containing Lidocaine (2.34 mg/ml, purchased from Astra Pharmaceutical Products, Inc.) as described by Mishell and Shiigi [12]. Cells were scraped from dishes, pooled and counted for future investigation.

Nonadherent (Nad) Cells: The method used by Letvin et al. [13] of passing spleen cells through Sephadex G-10 was followed to obtain an Ad cell-depleted population. Recovery was approximately 50%.

Antiserum treatment: Spleen cells at 10^7/ml in RPMI 1640 were treated with monoclonal anti-mouse Lyt-2.2 ascites fluid (IgM) according to instructions supplied by the vendor, New England Nuclear: 30 minutes at 37°C with antibody at 1/500, then for another 60 minutes at 37° on further dilution with rabbit complement (absorbed on mouse RBC) at final concentration of 1/6. Fetal calf serum (5%) was present in the medium throughout the treatment period. After two washings, cells were brought back to the starting volume without being recounted.

Statistics: Student's t test was used to assess significance between values. A p value equal to or less than .05 was considered to indicate a significant difference between two means.

RESULTS

The first series of experiments was designed to examine O_3 effects after continuous exposure of mice for periods of up to four weeks. Groups (3-5 mice each) were removed at weekly intervals from the three basic sets (O_3, Sham, and N), and after having body weights recorded, were sacrificed for further study. Not too surprisingly, mice that had been in O_3 had lost considerable body and thymus weight by the end of a week, as shown in the top half of Table 1. That the loss of thymus weight did not simply reflect the stress of living in an exposure chamber was apparent from the similar weights of the Sham group and the normal control group (N) throughout Table 1. Additional confirmatory data (not presented here) were obtained at three and four weeks in another study.

When daily measurements were made throughout the first week of exposure in another series of observations (second half of Table 1), thymus weights were significantly decreased in the O_3 group by day 2 and body weights by day 3. Again, the Sham and N groups did not show significant differences.

Among several parameters measured in the series of experiments represented above and in two others not described was spleen cell response to the mitogens Con A, PHA, and LPS. No consistent, specific effect of O_3 was seen, despite care taken to assay within appropriate ranges of cell and mitogen dose. Data are presented in Table 2 where it can be seen that at one week responses to Con A and LPS appear to be decreased in both O_3 and Sham groups. No differences were seen at later times.

Table 3 illustrates findings relative to ability of experimental mice to engage in primary and secondary <u>in vitro</u> immune responses to SRBC. It is very clear from values in the top half of the table that spleens from mice in the O_3 group showed a greatly decreased primary response as expressed in terms of plaques either per plate (12×10^6 cells) or per spleen. Data from the second series of experiments, while not showing a progressive, clear-cut change with time, did in general confirm a decline in the ability of O_3-exposed mice to engage in a primary response. No consistent change attributable to exposure conditions was seen in the secondary response to SRBC. Although O_3 values for secondary response at 2 of the 4 sample times were significantly different from N, one represented an increase, the other a decrease. Note also that signficant increases and decreases were measured from spleens of the Sham group.

To gain insight concerning the basis for a reduced primary response, the

experiment shown in Table 4 was carried out with spleen cells from controls and from mice exposed for 1 day to O_3. On the chance that suppressor cells were involved, mixing of cells from N and O_3 spleens was done, and in some cases anti Lyt-2.2 treatment was used on cells before culturing. There was clearly a decrease in the <u>in vitro</u> primary response from spleens of O_3 mice, to roughly half that of N spleens. When O_3 spleen cells were reacted with anti Lyt-2.2 antibody plus complement (O_3^{α}), with the expectation of removal of suppressor T lymphocytes, instead of finding the plaque number restored to that of N spleens, we recorded an even lower value. It is clear from this observation that the decreased primary response seen in O_3-exposed mice is not the simple effect of a suppressor cell bearing Lyt-2.2. The apparent decrease in plaques from O_3^{α} spleen cells compared to O_3 may represent a loss (cellular and/or functional) resulting from the long incubation at 37°C and from other manipulations required by antibody treatment. When N cells were mixed with O_3 cells at a ratio of 6:1, there was no indication of suppression. Instead, there was evidence that O_3 spleen cells were performing as well as N in this system. However, when O_3^{α} cells were added at the same ratio, many more plaques were found, the excess suggesting release from suppression. It can be concluded from the experiment in Table 4 that (a) O_3 mice have a decreased primary response (in confirmation of data presented in Table 3); (b) the primary response is not restored by removal of Lyt 2.2 positive cells; and (c) there is a suggestion from cell mixing experiments that 1 million O_3 cells are capable of more augmentation of the primary response of 6 million N spleen cells than are one million N or O_3 cells.

In order to determine whether adherent cells from O_3 mice were functioning normally in the Mishell-Dulton system, spleen cells from a 24-hour O_3 exposure

were separated into Ad and Nad populations and mixed together before culturing. Results, depicted in Table 5, show that in this particular case (as had been observed on occasion before) the O_3 spleen cells, unseparated, did not show a decreased primary response. There was a striking difference, however, between N and O_3 mice in the primary antibody formation derived from Nad cells. The Sephadex-passed populations were functionally altered, both of them decreased, N only slightly, in antibody forming capability below that of unseparated cells. When the separated populations (Ad and Nad) of N mice were mixed at a ratio of 0.9:9.1, only one quarter the number of plaques seen from Nad cells alone was recorded, probably reflecting a suppressive effect of macrophages or one of their soluble products. However, the addition of either N or O_3 Nad cells improved the response above the level resulting from O_3 Nad alone (compare 104 and 80 to 11). On the other hand, O_3 Ad cells, when mixed with N Nad cells, caused a further decrease in plaque number (compare 117 to 400). From these data it can be concluded that (a) Nad populations are lacking cells needed for optimal in vitro primary response; (b) Ad populations in the proportion tested here are suppressive; and (c) separated O_3 spleen cells, both in Ad and Nad fractions, appear to be defective.

Hemopoietic precursors were assayed in vivo (CFU-S) and in vitro (CFU-GM, CFU-E, BFU-E, and BFU-ME). Throughout these studies, although significant differences were measured on several occasions, no consistent pattern of change as a result of O_3 exposure was seen in ability of marrow to produce spleen colonies (CFU-S) in heavily irradiated isogenic recipients, as illustrated in Table 6. For example, the O_3 value for day 21 is significantly lower than that of the normal control in experiment P but not different in experiment E, where the standard errors were unaccountably large. Had the difference between 20.7

and 29.3 been significant in that case, the effect would have been in the direction opposite to that seen in Experiment P. In several but not all cases in which the O_3 values for colonies/10^5 cells appear to be different from N controls, Sham values are also significantly different from N. When the data are considered in terms of CFU-S harvestable from one tibia, there is suggestive evidence of a decrease in the O_3 group at 2 and 3 weeks (experiments D and E), and there were significant decreases in both values (content and concentration) at 4 weeks in Experiments C and Q. It is also interesting to note that variability was greater (i.e., range of colonies/10^5 cells for O_3: 15.7 to 42.0 and for Sham: 21.3 to 39.7) in animals kept in chambers than in mice maintained in the conventional animal facility (N: 20.7 to 32.0).

The early erythroid progenitor, BFU-E, measured by 7-day development of bursts of benzidine positive colonies, was found to be increased significantly for the first two weeks of a 3-week exposure as shown in Table 7. The second series of experiments produced values that varied widely from one assay to the next within the first few days of exposure, but on days 6 and 7 the same increase was seen as had been recorded previously for the O_3 group at days 7, 14, and 21. The fact that wide fluctuations were seen in all groups, including N controls, in the first few days of the second series of experiments (bottom half of Table 7) suggests that there were undefined problems with the BFU-E assay itself. Additional cultures were carried out on these marrow populations to evaluate BFU-ME. Although even more variable than the data in Table 7, BFU-ME results (not presented here) confirmed an increase in early erythroid precursors, by implication a stem cell common to megakaryocytes and CFU-E. Because the change in BFU-E concentration seen in the first series necessarily implied alterations in red cell production, hematocrits were measured on blood

from all mice in the second series. They are presented in Table 8 where it can be seen that, in values for the O_3 group there is a significant increase, appearing as early as day one and lasting for the entire week. This may be related to the fact that the O_3-exposed animals drank very little water (qualitative observation) during this period.

DISCUSSION

One reasonably consistent, positive finding in these studies was a decreased ability of spleen cells from O_3-exposed mice to engage in an *in vitro* primary immune response (see Table 3). An immune system so compromised constitutes a grave threat to the health of an animal whose lungs, already seriously injured by O_3 exposure, cannot serve as a natural barrier to airborne infections. Our attempt to deal with the question of whether a suppressor cell was present in O_3 spleens and could therefore account for the defective *in vitro* primary immune response, was complicated by the finding (see Table 4) of two contradictory answers. First, a simple mixing of O_3 putative suppressors with N spleen cells gave no suppression. Second, removal of Lyt-2.2 positive cells, among which should have been most suppressors, did not enable the remaining spleen cells to regain their primary response ability. On the other hand, when a million O_3^{α} cells were mixed with 6 million N cells, augmentation greater than that recorded for N + N or N + O_3 was seen. These findings show that a simple suppressor cell explanation is not adequate, and they suggest that a more complex interaction among several cell types - helpers, suppressors, and accessory cells or macrophages - has been altered by O_3 exposure.

The separation of spleen cells into Ad and Nad populations for testing in the Mishell-Dutton primary antibody response system produced data showing that a 24-hr whole body exposure to O_3 caused remarkable changes in the two fractions. Cells necessary for a successful primary response were severely depleted in the case of O_3 exposed mice by passage through Sephadex G-10, and Ad cells recovered from petri dishes were found to be more inhibitory than those separated by adherence from N mice. It is interesting also that O_3 Ad cells appeared to reconstitute the O_3 Nad population to some degree, but suppressed antibody formation in the Nad cells of N mice.

The other positive finding in our studies was an elevated tibial BFU-E concentration in O_3 mice. The basis for this, especially in the light of no consistent changes in CFU-E, CFU-GM, or CFU-S, is far from clear. In one series of experiments (labeled with the letters G-M in Table 8), hematocrit values demonstrated hemoconcentration in O_3 mice during the first week of exposure. Because BFU-E, in comparison with CFU-E, are less sensitive to erythropoietin [14], one might postulate that they (BFU-E) accumulate in response to a lowered EP level, which in turn would be the expected result of an increased hematocrit. It is difficult to interpret our BFU-E data in the light of findings from protein deprivation [15] and dehydration [16] experiments, inasmuch as the O_3 mice here had free access to food and water.

One of the most striking findings during the course of these experiments was the extreme variability of data from all assays. Not only were standard errors large within an experiment, but in some cases a second study done under identical conditions, to the best of our knowledge, gave a different answer

from the first, i.e., a significant effect one time but not the other. Inasmuch as exposure conditions were well controlled and carefully monitored, and the individual assay methods were ones with which we had had previous experience, it is likely that the variability reflected real differences among mice in their responses to treatment. If one takes into account the variability from mouse to mouse in response to the O_3-produced initial lung injury and then in response to resultant physiological change, such as endocrinological ones [17], it is perhaps not surprising that several of our assays failed to give consistent answers. The phenomenon of tolerance to O_3, described by early investigators in this field [18,19], could also have contributed to the extent of our standard error insofar as individual mice may have varied widely in ability to adapt physiologically. Our studies, in contrast to those investigating tolerance, involved continuous exposure except for very brief periods to allow washing of chambers and changing of food and water. We can only speculate, therefore, on analogies to tolerance, but we know that different degrees of physiologic adaptation must occur under these circumstances. Our variability was thus further compounded by the standard practice of pooling cells from at least 3 to as many as 8 animals for any given assay.

In summary, these data show (a) an inherent variability in biological response to ozone, (b) alteration in primary in vitro immune response, and (c) changes in erythroid progenitors in the bone marrow as a result of exposure. These results suggest that the deleterious effects of ozone inhalation extend beyond the lung, already known to be affected, to other organ systems to constitute a substantial threat to human health.

REFERENCES

1. L.D. Scheel, O.J. Dobrogorski, J.I. Mountain, J.L. Svirbely and H.E. Stokinger, Physiologic, biochemical, immunologic, and pathologic changes following ozone exposure. J. Applied Physiol., 14 (1959) 67.
2. D.L. Coffin, D.E. Gardner, R.S. Holzman and F.J. Wolock, Influence of ozone on pulmonary cells. Arch. Environ. Health, 16 (1968) 633.
3. M.L. Peterson, S. Harder, N. Rummo and D. House, Effect of ozone on leukocyte function in exposed human subjects. Environmental Research, 15 (1978) 485.
4. S. Miller and R. Erlich, J. Infect. Dis., 103 (1958 145.
5. A.L. Ibrahim, Y.C. Zee and J.W. Osebold, The effect of ozone on the respiratory epitheluim and alveolar macrophages of mice. 1. Interferon production. Proc. Soc. Exp. Biol. Med., 152 (1976) 483.
6. Committee on Medical and Biological Effects of Environmental Pollutants: Ozone and Other Photochemical Oxidants, Chapter 8, "Toxicology." National Academy of Sciences, Washington, DC, 1977, p. 323.
7. J.E. Till and E.A. McCulloch, A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat. Res., 14 (1961) 213.
8. A. Urabe and M.J. Murphy, Jr. in M.J. Murphy, Jr. (Ed.), In Vitro Aspects of Erythropoeisis, Springer-Verlag, NY, 1978, p. 28.
9. D.L. McLeod, M.M. Shreeve and A.A. Axelrad, Culture systems in vitro for the assay of erythrocytic and megakaryocytic progenitors, in M.J. Murphy, Jr. (Ed.), In Vitro Aspects of Erythropoiesis, Springer-Verlag, NY, 1978, p. 31.
10. D.L. McLeod, M.M. Shreeve and A.A. Axelrad, Chromosome marker evidence for bipotentiality of BFU-E. Blood, 56 (1980) 318.
11. C.C. Stewart, Murine mononuclear phagocytes from bone marrow, in D.O.

Adams, P.J. Edelson, H.S. Koren (Eds.), Methods for Studying Mononucleas Phagocytes, Academic Press, NY, 1981, p. 5.

12. B.B. Mishell, S.M. Shiigi (Eds.), Selected Methods in Cellular Immunology. WH Freeman and Company, 1980.

13. N.L. Letvin, M.I. Greene, B. Benacerraf and R.N. Germain, Immunological effects of whole-body ultraviolet irradiation: selective defect in splenic adherent cell function in vitro. P. N. A. S., 77 (1980) 2881.

14. C.J. Gregory, Erythropoietin sensitivity as a differentiation marker in the hemopoietic system: studies of three erythropoietic colony responses in culture. J. Cell Physiol., 89 (1976) 289.

15. W. Fried and A. Anagnostou, The role of protein and other nutritional factors in the regulation of erythropoiesis. In: Current Concepts in Erythropoiesis. C.D.R. Dunn (Ed.), John Wiley and Sons, Ltd, NY, 1983, p. 233.

16. C.D.R. Dunn and L.N. Smith, The effect of dehydration on erythroid progenitor cells in mice. Exp. Hematol., 8 (1980) 620.

17. E.J. Fairchild, II, S.L. Graham, M. Hite, R. Killens and L.D. Scheel, Changes in thyroid I^{131} activity in ozone-tolerant and ozone-susceptible rats. Toxicology and Applied Pharmacology, 6 (1964) 607.

18. H.E. Stockinger, Evaluation of the accute hazards of O_3 and oxides of nitrogen. Arch. Environ. Health, 15 (1957) 181.

19. H.E. Stockinger, L.D. Scheel, Ozone toxicity: immunochemical and tolerance-producing aspects. Arch. Environ. Health, 4 (1962) 327.

Table 1. Body and thymus weights[+] of O_3-exposed mice

Experiment	days of exposure	N body wt g	N thymus wt mg	Sham body wt g	Sham thymus wt mg	O_3 body wt g	O_3 thymus wt mg
B	7	30.5 ± 0.8	39.5 ± 2.9	30.3 ± 1.2	34.8 ± 1.8	21.2* ± 1.7	5.2* ± 0.6
F	7	27.8 ± 0.6	33.4 ± 1.3	27.5 ± 0.3	34.6 ± 1.5	22.2* ± 0.7	7.6* ± 0.6
A	14	31.2 ± 1.2	33.2 ± 3.8	29.3 ± 0.7	34.4 ± 2.8	22.5* ± 0.6	13.3* ± 1.6
D	14	28.0 ± 0.8	40.0 ± 2.6	27.3 ± 0.8	36.3 ± 3.4	21.3* ± 0.6	7.8* ± 1.0
E	21	30.0 ± 0.6	37.2 ± 2.5	29.2 ± 0.6	39.3 ± 1.2	20.8* ± 0.6	22.6* ± 3.7
C	28	30.5 ± 0.8	37.0 ± 1.2	31.7 ± 0.8	35.4 ± 2.9	17.8* ± 0.3	3.4* ± 0.2
J	1	29.6 ± 0.4	31.1 ± 0.9	31.3 ± 0.7	29.6 ± 3.7	27.5 ± 1.2	21.2* ± 1.5
G	2	28.9 ± 0.8	30.9 ± 4.5	29.0 ± 0.6	34.8 ± 1.1	26.5 ± 1.1	13.9* ± 0.7
H	3	29.6 ± 1.0	38.7 ± 2.4	29.7 ± 0.3	34.3 ± 2.5	24.9* ± 0.7	11.3* ± 1.0
M	4	34.6 ± 0.8	27.3 ± 1.7	31.3 ± 1.3	35.8* ± 1.7	24.6* ± 0.6	9.6* ± 1.2
L	5	32.6 ± 0.9	27.6 ± 2.4	31.6 ± 0.8	30.0 ± 1.4	26.2* ± 1.0	8.8* ± 0.5
I	6	28.9 ± 0.6	30.5 ± 2.1	29.8 ± 0.3	33.8 ± 2.5	26.0* ± 0.7	10.8* ± 1.7
K	7	30.4 ± 0.9	35.8 ± 1.1	31.5 ± 0.5	34.9 ± 2.1	27.1* ± 0.3	9.8* ± 0.6

[+] average of 3-5 observations ± standard error.

* significantly different from corresponding N value.

Table 2. Mitogen responsiveness of spleen cells from O_3-exposed mice

days of exposure	group	nil*	Con A+	PHA+	LPS+
7	N	3321 ± 484	20831 ± 1070	36519 ± 5822	21319 ± 1406
	Sham	1347 ± 83	7326++ ± 598	12684 ± 1635	11179++ ± 893
	O_3	1131 ± 99	6958++ ± 606	15437 ± 942	5917++ ± 390
21	N	2590 ± 405	15555 ± 1356	5372 ± 322	10736 ± 405
	Sham	2244 ± 36	14018 ± 1480	ND	7547 ± 1080
	O_3	2606 ± 414	8750 ± 530	6208 ± 452	14411 ± 781
28	N**	9542 ± 1550	22375 ± 4611	51854 ±14423	15805 ± 2079
	Sham	10450 ± 490	29703 ± 3918	61833 ± 2810	11857 ± 1392
	O_3	9013 ± 1437	16610 ± 2733	29641 ± 3887	12009 ± 998

* Unstimulated control cultures; mean of triplicate cultures ± SE in all cases.

** All cells plated @ 1.5 X 10^6/ml.

\+ Optimum mitogen doses used: Con A, 2.5 µg/ml; PHA, 2.5 µg/ml; LPS, 100 µg/ml.

++ Significantly different from N.

ND No data due to technical error.

Table 3. Effect of O_3 exposure on primary* and secondary** immune response

Expt	Exp. days		N /dish	N /spleen	Sham /dish	Sham /spleen	O_3 /dish	O_3 /spleen
B	7	Primary	2406	12880	3000	13375	49+	46
		Secondary	3772	34891	2560+	18560	2253+	3240
D	14	Primary	984	5601	908	3552	127+	515
		Secondary	341	2188	387	5611	564	8213
E	21	Primary	901	3915	1625+	9409	587+	1798
		Secondary	793	21088	853	25728	2220+	36046
C	28	Primary	861	2830	323+	1952	2+	1
		Secondary	3638	40085	946+	11623	4720	11092
J	1		540	2117	507	2953	32+	110
G	2		1040	6954	1195	7560	135+	343
H	3		540	2117	454	2208	454	1333
M	4		585	5298	918	5485	927+	2972
L	5		1452	9850	452+	3217	250+	1128
I	6		890	5776	1280+	8512	883	4253
K	7		806	4240	1433+	9990	751	2838

* IgM plaques after 4 days' in vitro incubation; 12 X 10^6 ACT-treated spleen cells/dish.

** IgM + IgG plaques after 3 days' in vitro incubation; 8 X 10^6 ACT-treated spleen cells/dish.

\+ Significantly different from N. Standard errors omitted from table for clarity of data presentation; usual error approximately 8% of mean.

Table 4. Effect of coculturing cells from different spleen donors on primary antibody formation

Spleen cells donor	number*	cultured with donor	number	IgM plaques/dish** measured	predicted+	measured X 100 predicted
N	6	---	---	1337 ± 68	---	---
	1	---	---	85 ± 14	---	---
O_3	6	---	---	602++ ± 39	---	---
	1	---	---	43 ± 18	---	---
O_3^{α}	6	---	---	195++ ± 12	---	---
	1	---	---	8 ± 4	---	---
N	6	N	1	1928 ± 106	1422 ± 82	135.6
N	6	O_3	1	1903 ± 124	1380 ± 86	137.9
N	6	O_3^{α}	1	3082++ ± 150	1345 ± 72	229.1

* In millions added per dish.

** IgM plaques. Mean ± S.E. of 6 observations on triplicate cultures (3 plaqued at 50 µl and 3 at 100 µl/slide).

\+ Plaques predicted on basis of simple addition of yields from separate cultures.

++ Significantly different from N or N + N, as appropriate.

$^{\alpha}$ Treated with anti Lyt-2.2 serum (NEN).

Table 5. Effect of O_3 exposure on function of adherent cells in Mishell-Dulton primary antibody formation

culture number	spleen cells	population*	X 10^{-6} number plated	plaques** plate	plaques** per 10^6 cells
1	N	none	10.0	5596 ± 327	560+ ± 33
2	N	Nad	5.5++	2195 ± 127	401+ ± 23
3	O_3	none	10.0	5500 ± 423	550+ ± 42
4	O_3	Nad	4.2++	45 ± 28	11+ ± 7
5	N N	Nad Ad	9.1 .9	1133 ± 77	113+ ± 8
6	N O_3	Nad Ad	9.1 .9	117 ± 13	12+ ± 1
7	O_3 N	Nad Ad	9.1 .9	1040 ± 107	104+ ± 11
8	O_3 O_3	Nad Ad	9.1 .9	797 ± 179	80+ ± 18

* See materials and methods for separation procedures. Percentage recovery: N, Ad = 24.1, Nad = 54.8; O_3, Ad = 15.5, Nad = 41.8.

** IgM plaques. Mean ± SE from 6 observations on triplicate cultures (3 plaqued at 50 µl, 3 at 100 µl/slide).

+ For statistical tests carried out on values for plaques/10^6 cells, the following were significantly different: 1 and 2, 1 and 4, 1 and 5, 2 and 5, 3 and 7, 3 and 8, 4 and 7, 4 and 8, 5 and 6, 6 and 8; the following were not different: 1 and 3, 5 and 7, 7 and 8.

++ Number of cells recovered from input of 10 million.

Table 6. Effect of O_3 exposure on tibial content and concentration of CFU-S

Experiment	days of exposure	Colonies/10^5 cells* (colonies/tibia) N	Sham	O_3
B	7	31.1 ± 1.5 (2147 ± 103)	30.4 ± 1.5 (2370 ± 117)	29.6 ± 2.7 (1745 ± 158)
F	7	ND**	39.7 ± 1.5 (2263 ± 70)	42.0 ± 1.3 (2688 ± 83)
A	14	30.3 ± 1.6 (1962 ± 103)	24.8$^+$ ± 0.9 (2062 ± 75)	22.6$^+$ ± 0.7 (2152 ± 67)
D	14	29.6 ± 1.9 (2093 ± 194)	33.0$^+$ ± 1.7 (1518 ± 79)	25.6$^+$ ± 2.1 (1331 ± 112)
E	21	20.7 ± 3.3 (2049 ± 390)	29.1 ± 3.8 (2188 ± 287)	29.3 ± 2.8 (1963 ± 190)
C	28	32.0 ± 1.6 (2198 ± 137)	33.5$^+$ ± 2.3 (2613 ± 156)	24.8$^+$ ± 0.8 (1463$^+$ ± 59)
P	21	24.7 ± 1.2 (2242 ± 108)	21.3 ± 3.1 (1685$^+$ ± 250)	16.6$^+$ ± 4.2 (1450$^+$ ± 363)
Q	28		21.3 ± 2.7 (1387$^+$ ± 173)	15.7 ± 2.0 (1347 ± 172)

$^+$ significantly different from N; number of observations/value reported = 6-13.

* mean ± SE.

** ND: not done.

Table 7. Effect of O_3 exposure on BFU-E concentration

		BFU-E/10^5*		
	Expt	exposure	N	ShamO_3
B	7	33.8 ± 8.5	27.9 ± 3.3	56.7 ± 6.3
F	7	36.7 ± 3.0	33.9 ± 2.2	58.1$^+$ ± 4.4
A	14	20.6 ± 2.6	25.2 ± 2.2	33.7$^+$ ± 2.0
D	14	32.1 ± 1.3	43.8$^+$ ± 3.2	66.1$^+$ ± 4.4
E	21	27.5 ± 2.7	27.3 ± 2.7	31.0 ± 3.0
J	1	34.2 ± 1.3	39.7 ± 3.2	37.0 ± 3.9
G	2	25.8 ± 1.3	45.3$^+$ ± 2.9	28.7 ± 1.1
H	3	35.2 ± 2.4	93.2$^+$ ± 3.3	51.7$^+$ ± 2.3
M	4	68.2 ± 5.0	32.7$^+$ ± 1.9	96.7$^+$ ± 4.8
L	5	15.0 ± 2.0	50.7$^+$ ± 3.2	41.5$^+$ ± 3.0
I	6	43.8 ± 3.1	42.0 ± 2.9	75.0$^+$ ± 2.0
K	7	42.2 ± 1.7	51.8 ± 5.4	79.6$^+$ ± 3.7

* Mean ± S.E.

$^+$ Significantly different from N.

Table 8. Effect of O_3 exposure on hematocrit

Expt	exposure	hematocrit* N	Sham	O_3
J	1	49.1 ± 0.3	48.0 ± 0.8	52.8 ± 0.8
G	2	49.1 ± 0.7	49.8 ± 0.4	56.2+ ± 0.9
H	3	50.0 ± 1.0	49.6 ± 0.5	52.6 ± 0.9
M	4	46.8 ± 0.3	47.0 ± 0.5	51.9+ ± 1.2
L	5	48.7 ± 0.5	49.2 ± 0.5	50.9+ ± 0.5
I	6	50.6 ± 0.8	49.8 ± 0.7	52.0 ± 1.2
K	7	49.2 ± 0.5	50.4 ± 0.4	52.0+ ± 0.5

* Mean ± S.E., 5 mice per group.

+ Significantly different from N.

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720