Mutation Caused by Human Phagocytes

Abstract. *Histidine-requiring mutants of* Salmonella typhimurium *TA100 were incubated with human peripheral blood leukocytes. More of these bacteria reverted to histidine independence than controls not incubated with cells. Phagocyte-rich suspensions were mutagenic, while heat-killed cells, lymphocytes, or mixed blood leukocytes of a patient with chronic granulomatous disease were not. Production of reactive oxygen metabolites could explain the capacity of phagocytes to induce mutation.*

Phagocytic leukocytes can be toxic to tumor cells (*1*) and there is evidence that phagocytes are important in defending against malignancy (*2*). Efforts to stimulate an immune or inflammatory response have yielded positive results, with improved survival in many tumor systems. However, certain chronic inflammatory conditions (ulcerative colitis, for example) are associated with leukocyte infiltration and a markedly increased risk of cancer in the affected tissue (*3*). Furthermore, metabolically active phagocytic leukocytes generate products that are potentially carcinogenic. In addressing this latter process, we provide evidence that human leukocytes have the ability to induce mutation.

To study mutagenesis, we used the microbial assay developed by Ames *et al.* (*4*). In this system histidine-requiring auxotrophs of *Salmonella typhimurium* are used; the types of mutations engineered into these strains are well characterized (*4*). Mutation at the histidine locus is determined by reversion to histidine independence. Previous studies revealed a high correlation between substances found mutagenic in this system and carcinogenic in animals (*4*).

Leukocytes were isolated from anticoagulated human blood by dextran sedimentation under sterile conditions (*5*). In some experiments, mononuclear cells were separated from granulocytes by means of Ficoll–sodium diatrizoate gradients (*5*). The mononuclear cell fractions contained approximately 75 percent lymphocytes and 25 percent monocytes when the suspensions were obtained from anticoagulated blood and more than 97 percent lymphocytes when they were prepared from defibrinated blood. Platelet contamination was negligible when defibrinated blood was used. Various concentrations of leukocytes were then added to the *Salmonella*-containing agar, as chemical mutagens would be. Hexose monophosphate shunt activity of the phagocytes, as demonstrated by oxidation of [1-^{14}C]glucose to $^{14}CO_2$ (*5*), was induced by the bacteria. The release of $^{14}CO_2$ in these incubations was four times greater than in control incubations of cells with bacteria-free agar.

The mutagenic activity of the leukocytes was demonstrated consistently with *Salmonella* strain TA100. Table 1 illustrates results obtained over a wide range of leukocyte concentrations with blood from several donors. There was considerable day-to-day variation in the number of colonies induced to revert by a given quantity of cells, but mutation was consistently produced.

Although dose responses were seen in individual experiments (Fig. 1), a clear dose response is not evident in the pooled data. At least three factors may account for this: (i) there is a great deal of interassay variability in the Ames test (*6*), (ii) there is a certain amount of day-to-day variation in the metabolic activity of leukocytes prepared from different donors, and (iii) nonphagocytic or dead cells might quench toxic products in some cases. Cells killed by heating for 1 minute at 100°C, however, did not cause mutation; this suggests that nonspecific products of cellular decomposition are not an important component of revertant induction. *Salmonella* strains TA98 (Fig. 1) and TA94 were also mutated by phagocytes.

The data for experiments in which fractionated leukocytes were used are given in Table 2. Mixed leukocyte suspensions (75 to 80 percent neutrophils) were the most effective at causing mutation, but mononuclear suspensions containing monocytes and lymphocytes also induced more revertants than control suspensions. Lymphocyte suspensions, on the other hand, were not mutagenic. This suggests that the cells most responsible for mutation are neutrophils and monocytes (that is, phagocytes).

It is possible to postulate a single

Table 1. Influence of leukocyte suspensions on the mutation rate of *Salmonella typhimurium* strain TA100.

Number of cells added	Number of revertant colonies	Number of determinations
0	136 ± 7*	22
10^5	200 ± 23	11
5×10^5	297 ± 63	10
8×10^5	221 ± 46	10
10^6	189 ± 18	13
2×10^6	159 ± 24	6
3×10^6	278 ± 95	3
(Heat-killed cells)	101 ± 9	6

*Spontaneous revertants.

Table 2. Influence of fractionated leukocyte suspensions on mutagenic activity. Counts for individual plates are given.

Number of cells added	Number of revertants
0*	98, 88, 103
Mixed leukocytes	
4×10^6	175, 211, 198
5×10^6	158, 277, 338
Mixed mononuclear cells	
4×10^6	112, 199, 200
5×10^6	104, 129, 124
0†	68, 88
Lymphocytes	
10^5	35, 64, 70
5×10^5	56, 69, 236
10^6	70, 51, 65
4×10^6	55, 65
5×10^6	49, 47

*Control condition for experiments with leukocytes and monocytes. †Control condition for experiments with lymphocytes.

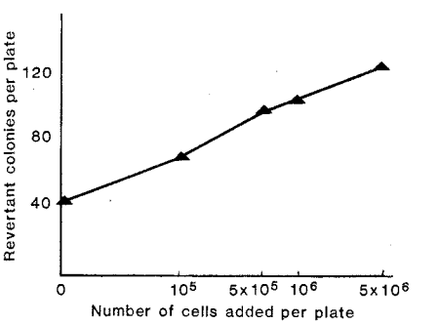

Fig. 1. Mutation response of *Salmonella typhimurium* TA98 to increasing concentrations of leukocytes. Each point represents the mean of triplicate determinations.

Table 3. Comparison of mutagenicity of normal leukocytes with that of leukocytes from a 19-year-old CGD patient in *Salmonella* strain TA100. The diagnosis of CGD was established by the patient's history of recurrent infections due to *Staphylococcus aureus* and by failure of his peripheral leukocytes to reduce nitro blue tetrazolium or generate superoxide anions in the presence of particulate or soluble stimuli (*7*). Each entry is the mean ± standard error of six determinations.

Number of cells added	Number of revertants
0	58 ± 22
Normal leukocytes	
10^6	290 ± 94
CGD leukocytes	
10^6	74 ± 34

0036-8075/81/0501-0546S00.50/0

mechanism whereby phagocytes could be either beneficial or harmful with respect to neoplasia. Products of phagocytic oxidative metabolism, such as superoxide anion, hydrogen peroxide, and hydroxyl radical can be lethal to tumor cells (*7*). Furthermore, lipids can be peroxidized by these reactive oxygen species, producing cytotoxic metabolites such as malonyldialdehyde (*8*). However, these products are also capable of interacting with DNA and producing mutations (*9*). To test our hypothesis further, we compared the mutagenicity of neutrophils from a patient with chronic granulomatous disease (CGD) with that of neutrophils from normal individuals. In CGD, neutrophils have a defect in the NAD(P)H oxidase-superoxide–generating system and are thus unable to generate superoxide and hydrogen peroxide. In other respects, they are normal phagocytes (*7*). As shown in Table 3, the mutagenicity of cells from the CGD patient was markedly diminished compared to that of normal cells.

These data indicate a major role for oxygen metabolites in the mutagenic process. Therefore, as in the case of ionizing radiation, it is likely that the seemingly antithetical effects of tumor cell toxicity and carcinogenesis are produced by the same basic mechanism. In particular, efforts to study the role of oxygen in mutagenesis produced by phagocytes should be pursued.

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10. We thank M. Grifoni and M. Desmond for their assistance and B. Ames for providing the bacteria. Supported by PHS grant CA 09321, the Edwin S. Webster Foundation, Edwin W. Hiam, Charles and Jane Kaufman, and the Tomasello Club.

4 December 1980

Transformation of Paralytic Shellfish Toxins as Demonstrated in Scallop Homogenates

Abstract. *Toxins in shellfish, which are responsible for paralytic poisonings, undergo reductive transformation when incubated with the homogenate of various portions of the scallop,* Placopecten magellanicus. *The transformation includes the reductive elimination of* O*-sulfate groups, a change that is most evident in the locomotor tissue homogenates. The commercially important adductor muscles can also inactivate the toxins.*

Paralytic poisons in shellfish present serious health and economic problems. They are also a spectacular manifestation of metabolite transfers prevalent among many marine organisms through the food chain.

Off the west coast of the United States, the toxic dinoflagellate, *Gonyaulax catenella*, is ingested by shellfish, and the toxic component, saxitoxin (**1**) accumulates in certain tissues of the shellfish (*1–4*). In other parts of the world, shellfish poisoning is more complex, involving several other toxins (*5, 6*). In 1978, we reported on the toxins found in toxic sea scallops from the Bay of Fundy, New Brunswick, Canada (*7*), and later another report gave a considerably different toxin profile on the scallops obtained from almost the same location (*8*). These conflicting results indicated the possibility of bioconversions of the toxins in the scallops.

Scallops, *Placopecten magellanicus*, collected in the Bay of Fundy in October 1977, were toxic. The adductor muscles

Table 1. Toxin contents in the homogenate of toxic scallops before and after incubation and in the toxin mixtures incubated with different body regions (gill, foot, visceral mass, and muscle) of nontoxic scallops in acetate buffer (*p*H 5.8) for 3 days. Recovery of toxicity was calculated as 100 times the total toxicity after incubation divided by the total toxicity before incubation. Toxicity was determined by the method of the Association of Official Analytical Chemists (*18*); 1 MU is equivalent to 0.18 μg of saxitoxin dihydrochloride.

Toxin	Percent toxicity in scallops		Percent toxicity in toxin extracts incubated with			
	Before incubation	After incubation	Gill	Foot	Visceral mass	Adductor muscle
Gonyautoxin-IV + gonyautoxin-V	3.2	0	3.5	0	2.2	3.6
Gonyautoxin-I + gonyautoxin-III	30.0	13.8	29.0	13.9	22.3	15.3
Gonyautoxin-II	25.0	9.7	24.5	8.3	29.6	33.3
Neosaxitoxin	32.0	13.8	30.9	12.5	23.7	5.0
Saxitoxin	6.4	62.7	6.6	65.3	22.2	42.8
Recovery of toxicity		100	100	100	100	46.8

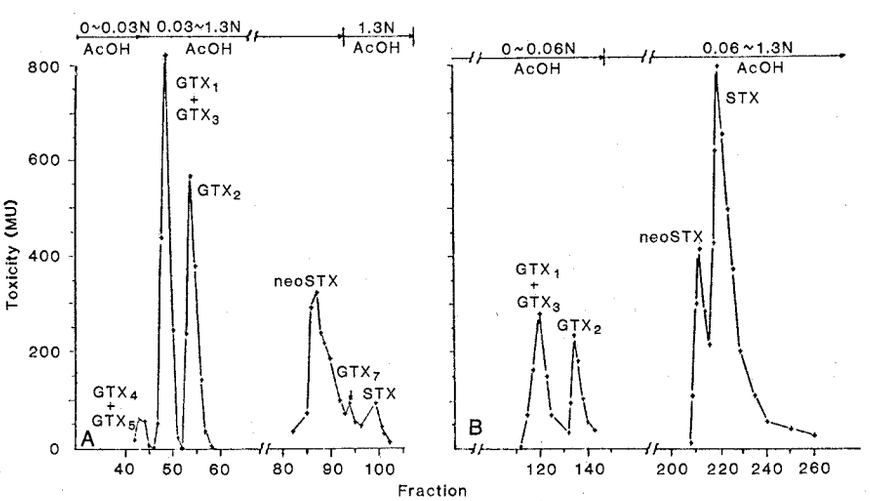

Fig. 1. Comparison of toxin patterns of toxic scallop homogenates (A) not incubated and (B) incubated. Fractions (7.5 ml each) were collected from Bio-Rex (1.5 by 110 cm) with use of the indicated buffer solutions (*7*). Toxicity level was expressed in mouse units (*MU*) (*18*). *GTX*$_1$ to *GTX*$_7$, gonyautoxin-I to gonyautoxin-VII; *STX*, saxitoxin; *neoSTX*, neosaxitoxin.

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