ular were occluded, eliminating countercurrent heat exchange, the cool carotid blood would gain heat from the surrounding brain tissue rather than jugular venous blood, thus cooling the brain. Heath (*6*) associated internal jugular occlusion with increased cephalic venous pressure, eye-bulging, and diminished head-to-body temperature gradient. DeWitt (*4*) observed that when the head of *Dipsosaurus* was heated the eyes protruded. He suggested that *Dipsosaurus* employed the same mechanism as *Phrynosoma* for equalizing head and body temperatures. Constriction of the internal jugular muscle at eccritic temperatures may result in the disappearance of the head-to-body temperature gradient. However, its constriction during panting could result in reversal of the gradient, keeping the brain cool relative to the rest of the body. It is possible, then, that the internal jugular constrictor muscle plays a more general thermoregulatory role in lizards than equalizing head and body temperatures.

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Tumor Immunity in vitro: Destruction of a Mouse Ascites Tumor through a Cycling Pathway

Abstract. *Lymphocytes in peritoneal exudate from BALB/c mice immunized against ascites leukemia EL4 are uniquely efficient at destroying ^{51}chromium-labeled EL4 cells in vitro. The lytic process depends upon the number of lymphocyte–tumor cell interactions. Effector lymphocytes are not inactivated as a result of lethal contact but can interact repeatedly with tumor cells.*

Lymphoid cells from an animal immunized against a tumor can bring about the destruction of the tumor in vitro. This immune process is believed to be analogous to cell-mediated tumor regression in vivo (*1*, *2*). In an attempt to elucidate the cell mechanism involved in tumor rejection, we studied in vitro the destruction of tumor cells by lymphocytes that had been anatomically associated with the rejection of an ascites tumor in vivo (*3*). We report that effector lymphocytes are not inactivated as a result of lytic contact with tumor cells but can recycle to interact with and destroy additional tumor cells. The rate of tumor cell destruction depends upon the number of lymphocyte-tumor cell interactions.

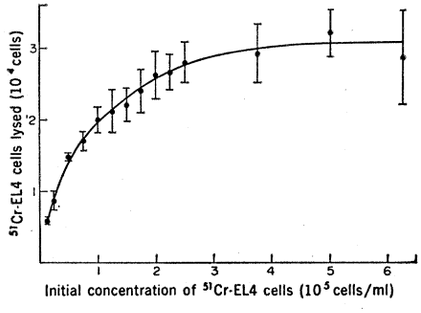

Fig. 1. Lysis of ^{51}Cr-EL4 cells as a function of ^{51}Cr-EL4 cell concentration. Lymphocytes (1×10^6) from BALB/c mice immunized with EL4 cells were mixed with the indicated concentrations of ^{51}Cr-EL4 cells and incubated while rocked for 45 minutes at 37°C. Control cultures contained normal BALB/c peritoneal lymphocytes under the same conditions. Results given were obtained by subtracting the means of triplicate control cultures from means of triplicate experimental cultures. Vertical bars indicate ranges.

Lymphoid cells were obtained from the peritoneal exudate of BALB/c mice 11 days after an intraperitoneal injection of 3×10^7 EL4 cells (*4*). The crude peritoneal exudate, consisting primarily of macrophages and small- and medium-sized lymphocytes, was passed through nylon wool columns to remove macrophages. The eluted nonadherent lymphoid cells (lymphocytes) were extremely effective at destroying tumor cells in vitro (*3*). In the cytotoxicity measurements, unlabeled lymphocytes and EL4 cells labeled with sodium [^{51}Cr]chromate (^{51}Cr-EL4 cells) were incubated in 1-ml portions of medium containing 10 percent fetal calf serum (*5*). The mixtures were rocked (5 cycle/min) at 37°C, and then the amount of radioactivity released was measured (*2*, *6*). The number of lysed ^{51}Cr-EL4 cells was determined by comparing the radioactivity released into culture supernatants with that obtained by freezing and thawing a known number of ^{51}Cr-EL4 cells.

To determine whether tumor cell destruction depended on the number of interactions between lymphocytes and tumor cells, and also to determine the subsequent fate of the lymphocytes, the number of tumor cells destroyed by a given number of lymphoid cells was measured.

In the first set of experiments, a constant number of immune lymphocytes was mixed with increasing numbers of ^{51}Cr-EL4 cells, and the number of tumor cells lysed was determined. In a 45-minute incubation, the number of ^{51}Cr-EL4 cells lysed increased as a function of the initial concentration of tumor cells (Fig. 1). However, the number of lysed cells reached a plateau when the initial concentration of ^{51}Cr-EL4 cells was 3×10^5 ml^{-1}. The number of ^{51}Cr-EL4 cells destroyed at this point (about 3×10^4) was the maximum number that could be lysed with the time interval and cell concentrations used (*7*). This number, however, did not reflect the total lytic capacity of the lymphocytes, because longer incubation resulted in more killing (Fig.

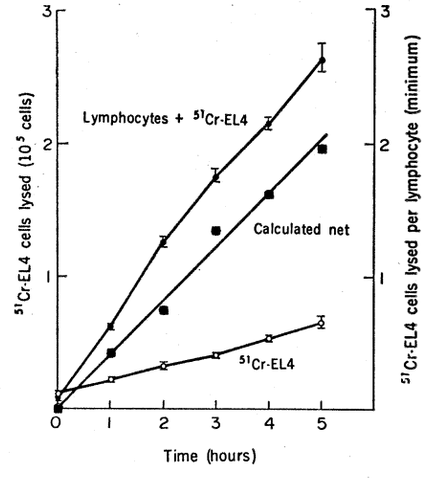

Fig. 2. Killing capacity of lymphocytes from BALB/c mice immunized with EL4 cells. Immune lymphocytes (1×10^6) were mixed with ^{51}Cr-EL4 cells (8×10^5) and incubated while rocked at 37°C for the times indicated. Results are means of quadruplicate cultures, and the vertical bars indicate ranges. The net number of ^{51}Cr-EL4 cells lysed (■) was calculated by subtracting mean control values (○, ^{51}Cr-EL4 cells) from mean experimental values (●, lymphocytes + ^{51}Cr-EL4 cells).

2). The suggestion (*8, 9*) that destruction of target cells by lymphocytes depends upon the number of lymphocyte–target cell interactions is further supported by other experiments which demonstrated that the extent of target cell destruction also depended on the concentration of lymphocytes (*3, 8–12*) and that rocking facilitated lysis (*6*).

In the second series of experiments, examination of the fate of lymphocytes after lytic interaction with tumor cells showed that the lymphocytes involved were not inactivated during the process but were capable of destroying additional tumor cells. For these experiments, 1×10^5 lymphocytes were mixed with 8×10^5 ^{51}Cr-EL4 cells, and the number of tumor cells destroyed was determined as a function of time. It was known from previous experiments (Fig. 1) that 8×10^5 represented a considerable excess of tumor cells. The number of tumor cells destroyed in 5 hours was twice the number of lymphocytes present, and there was no decay in the rate of destruction (Fig. 2). If the reacting lymphocytes were "consumed" or inactivated, there would have been a significant decrease in the rate of destruction, and the total number of tumor cells lysed would have been no greater than 1×10^5 (*13*). Although previous experiments led to the suggestion that the lymphocyte is not inactivated as a result of its cytolytic activity (*8, 12, 14*), this conclusion has recently been questioned by Henney (*13*). Under the conditions of our experiments, the lymphoid cell kills and continues to kill (*15*).

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4. Tumor EL4, obtained from the National Cancer Institute, Bethesda, Maryland, is an ascites leukosis carried in C57BL/Spr mice [P. A. Gorer, *Brit. J. Cancer* **4**, 372 (1950)]. The rejection by BALB/c hosts is usually complete by 11 days after transplantation.
5. Medium RPMI-1640 or phosphate-buffered saline (Grand Island Biological Co., Grand Island, New York) serves equally well for short-term experiments. Sodium [^{51}Cr]chromate (Amersham-Searle Co., Des Plaines, Illinois) was used for labeling as previously described (*2, 3, 6*).
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7. A higher concentration of lymphocytes (2×10^6 ml^{-1}) at this concentration of tumor cells (3×10^5 ml^{-1}) resulted in more lysis.
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13. It is believed, on the basis of quantitative kinetic analysis and microcinematography, that one lymphocyte is required and sufficient to destroy one target cell [Wilson (*10*); Berke *et al.* (*8*); H. Ginsburg, W. Ax, G. Berke, in *Pharmacological Treatment in Organ and Tissue Transplantation*, A. Bertelli and A. P. Monaco, Eds. (Williams & Wilkins, Baltimore, 1970), p. 85; C. S. Henney, *J. Immunol.* **107**, 1558 (1971)].
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15. By using ^{51}Cr-labeled lymphocytes, we recently obtained direct evidence that lymphocytes are not killed during the reaction.
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Gene Differences between Caucasian, Negro, and Japanese Populations

Abstract. *The numbers of gene (codon) differences per locus between two randomly chosen genomes within and between Caucasian, Negro, and Japanese populations have been estimated from gene frequency data for protein loci. The estimated number of gene differences between individuals from different populations is only slightly greater than the number between individuals from the same population.*

Nei (*1, 2*) developed a statistical method by which the number of gene (codon) differences per locus between two different populations can be estimated from gene frequency data. He also indicated that the number of codon differences per locus is an ideal measure of gene differences between populations. We have used this method to investigate the gene differences between the three major ethnic groups of man, Caucasoids, Negroids, and Mongoloids (Japanese). The results obtained indicate that the gene differences between individuals from different ethnic groups are only slightly greater than those between individuals from the same group.

The method used is based on the identity of genes within and between populations. Thus if x_i and y_i are frequencies of the ith allele at a locus in populations X and Y, respectively, the probability of identity of two randomly chosen genes is $j_X = \Sigma x_i^2$ in population X and $j_Y = \Sigma y_i^2$ in population Y. The probability of identity of two genes, chosen at random, one from each of the two populations, is $j_{XY} = \Sigma x_i y_i$. We designate by J_X, J_Y, and J_{XY} the arithmetic means of j_X, j_Y, and j_{XY} over all loci, including monomorphic ones, respectively. Then, a minimum estimate of the number of net codon differences per locus (D) between X and Y can be obtained by

$$D = D_{XY} - (D_X + D_Y)/2 \qquad (1)$$

where $D_X = 1 - J_X$ and $D_Y = 1 - J_Y$ are minimum estimates of the number of codon differences per locus between two randomly chosen genomes (intrapopulational codon differences) in populations X and Y, respectively, while $D_{XY} = 1 - J_{XY}$ is a minimum estimate of the number of codon differences per locus between two genomes, one from each of the two populations (*3*). Note that D_X, D_Y, and D_{XY} are equal to the expected proportions of different genes between two randomly chosen genomes from the respective populations. On the other hand, a maximum estimate of the number of net codon differences per locus is obtained by using $D_{XY} = -\log_e J_{XY}$, $D_X = -\log_e J_X$, and $D_Y = -\log_e J_Y$ in Eq. 1, where J_{XY}, J_X, and J_Y are the geometric means of j_{XY}, j_X, and j_Y, respectively (*4*). The real number of codon differences is expected to be somewhere

Table 1. Minimum and maximum estimates of the number of codon differences* per locus within and between Caucasian and Negro populations. The subscripts C and N refer to the Caucasian and Negro populations, respectively; D stands for the number of net codon differences between the two populations. These estimates are based on gene frequency data for 44 protein loci.

	D_C	D_N	D_{CN}	D
Minimum†	0.107	0.092	0.110	0.011
Maximum	.139	.112	.142	.017

* Codon differences that are detectable by electrophoresis. † Minimum estimate of codon differences per locus is equal to the expected proportion of different genes between two randomly chosen genomes.