ites were much larger than normal (Fig. 1, B and D). We also observed that if the synchronized parasites were exposed to the immune serum before segmentation, many failed to release their merozoites; thus reduction in the expected number of successful merozoite invasions may not be due entirely to merozoite-blocking antibody, but to a failure of schizonts to reach complete maturation.

Our results fully support the findings of Taliaferro and Taliaferro (*1*), who reported that the first indication of immunologic crisis in *P. brasilianum* infections was a loss of the characteristic parasite synchrony; this was accompanied by retardation of development and resulted in a reduced number of merozoites in the disintegrating intracellular parasites or segmenters. It would be difficult to observe crisis forms and intracellular deterioration of mature parasite stages in natural infections of *P. falciparum* because the trophozoite- and schizont-infected red cells are sequestered in capillaries and are rarely seen in peripheral circulation (*14*). Our findings are supported by a report that monoclonal antibodies to *P. falciparum* retard intracellular parasite development (*15*), but are at variance with several reports on the effects of immune serums on cultured parasites (*4*, *6*, *16*). Some of these investigations were conducted before the continuous cultivation of *P. falciparum* was achieved (*16*), and therefore the observations on dying cultures in vitro might not have given a true picture of the effects of immune serum. In other studies, current cultivation techniques were used with serums obtained from *Aotus trivirgatus* monkeys made refractory to infections with *P. falciparum* by repeated cycles of infection and drug treatment (*6*); these studies failed to demonstrate crisis forms and retardation of intracellular development of parasites, perhaps because the *Aotus* monkeys do not respond immunologically to *P. falciparum* infections as humans do.

We have demonstrated that immune serums collected from donors in the Sudan inhibit merozoite invasion into erythrocytes, as expected. In addition, these serums retard intracellular parasite development and lead to crisis forms and parasite disintegration. The inhibitory factors are apparently specifically acquired because serums from residents of Khartoum, who are of the same genetic stock as people in rural villages, are not inhibitory and do not retard parasite development. The most plausible explanation for the action of these immune serums is that they prevent the function of parasite antigens inserted into the erythrocyte membrane for the nutritional benefit of the developing parasite. Parasite antigens on the surface of infected erythrocytes have been described (*12*). The complexing of antibody to such antigens may block the uptake of essential nutrients and lead to retardation of parasite development. Since it has been generally accepted by investigators in malaria immunology that protective antibodies to malaria are directed against the merozoite, our results could lead to the discovery of protective antigens not associated with the merozoite.

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## HEL Cells: A New Human Erythroleukemia Cell Line with Spontaneous and Induced Globin Expression

Abstract. *A new human erythroleukemia cell line has been established. This line, designated HEL, is capable of spontaneous and induced globin synthesis, producing mainly Gγ and Aγ chains. Embryonic chains (ε, ζ) and α chains are detectable in very small amounts; β chains are undetectable. This line provides a new model system for studying aspects of erythroid cell differentiation and differential globin gene expression.*

Established cell lines are invaluable tools in cell biology. Murine hemopoietic cell lines, specifically the Friend erythroleukemia cells, have greatly expanded our knowledge regarding hemopoietic cell proliferation and differentiation and have been useful in studying gene expression. Recently, human hemopoietic cell lines established from patients with leukemia have become available (*1*); K-562 has been the only human cell line which expresses phenotypic markers of erythroid lineage (*2*) and displays spontaneous and induced globin synthesis (*3*).

We report here the establishment of a new human hemopoietic cell line which is capable of globin synthesis. This new cell line, designated HEL (for human erythroleukemia), was derived from the peripheral blood of a patient with Hodgkin's disease who later developed erythroleukemia. The patient received allogeneic bone marrow transplantation, but relapsed 7 months later. At the time of his relapse, Wright's Giemsa–stained blasts from peripheral blood and bone marrow resembled large abnormal proerythroblasts with basophilic agranular cytoplasm and showed a marked tendency for polyploidy. Their cytochemical characteristics (positive in periodic acid–Schiff reagent, acid phosphatase, and butyrase; negative in myeloperoxidase, chloroacetate esterase, and Sudan black B) were consistent with the diagnosis of erythroleukemia. Peripheral blood mononuclear cells from this patient at the time of relapse were used to initiate suspension cultures. Cells were cultured in


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RPMI 1640 medium containing 12 percent fetal calf serum and supplemented with 2 m*M* glutamine, 1 m*M* pyruvate, penicillin (1 U/ml), and streptomycin (1 μg/ml). Active cell growth was apparent 1 month later and the cells have been maintained in continuous culture ever since (now over 20 months) and have undergone more than 70 passages.

In suspension cultures HEL cells have a doubling time of about 24 hours in log phase growth and reach a saturation density of approximately $1 \times 10^6$ per milliliter. They can be cloned in semisolid media (methyl cellulose) with an efficiency of up to 40 percent.

The cells do not have characteristics of Epstein-Barr virus–transformed B-cell lines, since they are negative for Epstein-Barr virus nuclear antigen and have neither surface nor cytoplasmic immunoglobulin by immunofluorescence. Further analysis indicated that the HEL cells were derived from the patient's malignant blasts. Cytogenetic studies of the blasts in vivo disclosed a modal chromosome number of 66, the presence of two Y chromosomes in 86 percent of the cells, and two double minute chromosomes in 28 percent of the cells. Similar findings were observed in the cell line after 2 months and after 9 months in culture. The cytochemical findings in the HEL cell line were also similar to those of the patient's blasts. Further immunofluorescence studies showed that the HEL cells express HLA antigens, β-2 microglobulin, and, in a proportion of cells (10 to 15 percent), Ia antigens; common acute lymphoid leukemia antigen was not detected and the cells were negative for surface antigens characteristic of T-lymphoid cells (*4*). We detected Fc receptors in a high proportion of HEL cells (60 percent).

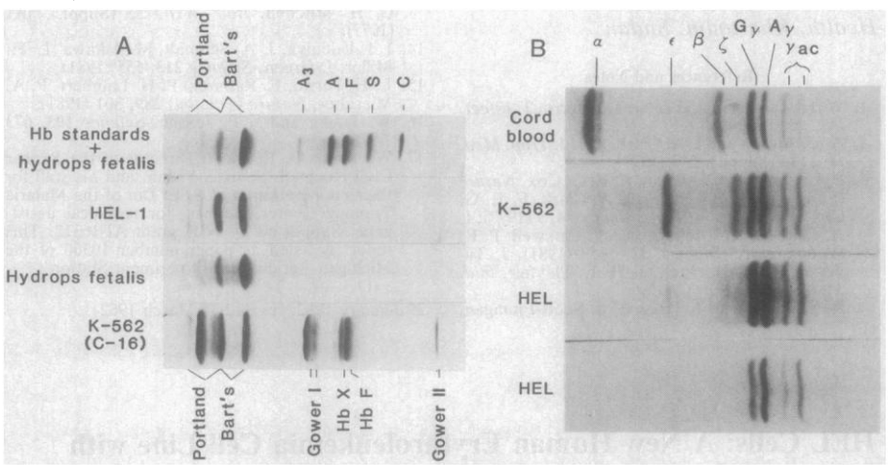


Fig. 1. (A) The hemoglobin phenotype of induced HEL cells is compared with the phenotypes of induced K-562 cells and the hemolyzate of a fetus with hemoglobin Bart's hydrops fetalis (homozygous α-thalassemia due to α-globin gene deletion); hemoglobin controls are also included for comparison. Large numbers of HEL cells or K-562 cells were induced, the cells lysed, and the lysate was used for hemoglobin isoelectric focusing (*5*). The gels were stained with benzidine. (B) Isoelectric focusing of globin chains in the presence of NP-40, urea, and β-mercaptoethanol was performed according to Righetti *et al.* (*6*) with slight modifications. Cell lysates were purified through haptoglobin binding (*10*) before isoelectric focusing and fluorography. A clear separation of ε and ζ chains from all species of γ chains and from β and α chains was achieved.

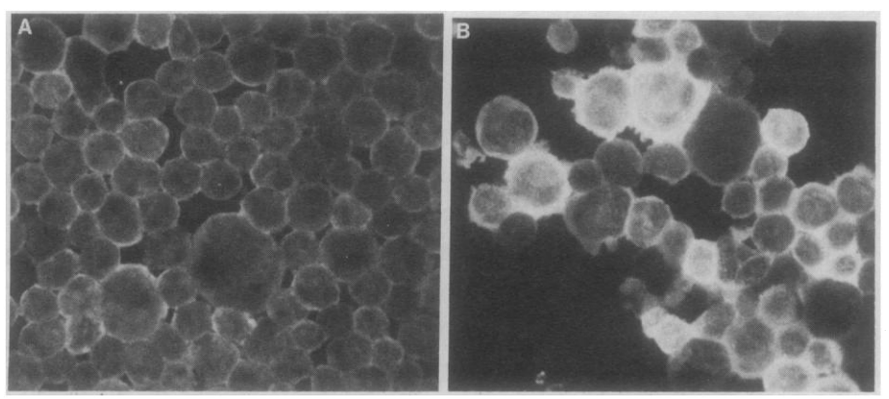


Fig. 2. Fluorescent labeling of HEL cells with a monoclonal antibody to γ chains conjugated to fluorescein isothiocyanate. (A) Cells before induction. (B) Cells after hemin induction. Globin accumulation in these cells was heterogeneous and unrelated to any morphologic changes induced by hemin. In both uninduced and induced cells the difference in cell size and the presence of large polyploid cells were characteristic.

Several studies were performed to test whether the cells could be induced to express erythroid-specific characteristics and whether they were capable of globin synthesis. Actively proliferating cells, with a density of $1 \times 10^5$ to $2 \times 10^5$ cells per milliliter were exposed to 50 μ*M* hemin for 4 days and then studied for globin expression and erythroid differentiation. As a preliminary approach, benzidine staining was performed, either in cell suspension or in fixed cell preparations. In uninduced cells, benzidine-positive cells were virtually absent, whereas after hemin induction benzidine-positive cells were frequently seen and their number increased as the hemin concentration was increased from 10 to 100 μ*M*. Definitive evidence for globin synthesis was provided by globin biochemical analysis and globin biosynthesis studies. Lysates from hemin-induced HEL cells were subjected to isoelectric focusing (*5*) in polyacrylamide gels, along with concurrent hemoglobin controls and lysates from K-562 cells (Fig. 1A). HEL cells produce mainly hemoglobin Bart's (Hb $\gamma_4$) and only trace amounts of other embryonic hemoglobin species. HbA was not seen in these or in subsequent studies. Analysis of globin chains from HEL cell lysates, by means of isoelectric focusing (*6, 7*) followed by fluorography (Fig. 1B), confirmed the findings from hemoglobin gels; the major species of globin chains synthesized by the HEL cells were the γ chains (Gγ, Aγ, and their acetylated equivalents) with a slight predominance of Aγ synthesis compared to Gγ. The same pattern of globin chain synthesis was also observed when lysates from uninduced HEL cells were studied. Quantitative studies indicated a tenfold increase in globin synthesis after hemin induction. Thus induction of HEL cells by hemin is associated with quantitative rather than qualitative changes in globin chains.

The presence of γ chains in uninduced and induced HEL cells was confirmed by immunofluorescence studies in which we used a fluorescein–conjugated monoclonal antibody specific for γ chains (*8*). Whereas uninduced HEL cells showed only a rare fluorescent cell after labeling with the γ-chain antibody, after exposure to 20 μ*M* hemin for 3 days nearly half of the cells were positively stained

(Fig. 2). Exposure to higher concentrations of hemin (50 $\mu M$) increased the proportion of labeled cells (up to 80 percent), but was accompanied by increasing signs of toxicity (decrease in proliferative rate and proportion of live cells).

The pattern of globin synthesis by HEL cells raises several questions. It is unclear at present why only the $\gamma$ chains are expressed and why the $\beta$ chains are absent. Further studies in which molecular approaches are used could provide insights regarding the expression of genes of the $\beta$-globin genomic region as well as the molecular basis of the severe $\alpha$-thalassemia phenotype in HEL cells.

It is also of special interest that this new human erythroleukemic line expresses characteristics associated not only with erythroid lineage but with other, nonerythroid, lineages (*9*). Whether this is a consequence of neoplastic transformation or whether it suggests that these particular cells are multipotent remains to be established. Further studies on the HEL cells may elucidate the relation between the phenotypic characteristics of these cells and differential globin gene expression.

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## Tumorigenesis at a Predetermined Oral Site After One Intraperitoneal Injection of *N*-Nitroso-*N*-Methylurea

Abstract. *Tumors in the soft tissues of the oral cavity of rats developed at predetermined sites as a result of a combination of an intraperitoneal injection of a direct-acting carcinogen,* N-*nitroso*-N-*methylurea, and a continuous irritation of the buccal mucosa by a stainless steel wire. The incidence of histologically malignant tumors was significantly higher in the irritated area than in any other area of the body. These results constitute evidence for a carcinogenic mechanism whereby the cells that develop into tumors may require the promotional effect of a nonspecific, nonmutagenic stimulus.*

Several investigators have reported on the production of tumors in the soft tissues of the oral cavity by carcinogenic agents applied topically for extended periods of time (*1*). The fundamental premise on which these experiments are based is the requirement that the carcinogen be in direct contact with the cells that will eventually form the tumor. Although supporting evidence in this regard has been published, absolute certainty for this inference is lacking (*2*). The purpose of our study was to determine if a single intraperitoneal injection of a carcinogen could predictably stimulate tumor formation at a predetermined site on the buccal mucosa that was continuously irritated by a stainless steel wire.

We used 116 male Wistar/Furth (W/F) rats, 6 to 8 weeks old, in these experiments (Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts). The carcinogen *N*-nitroso-*N*-methylurea (NMU) (ICN Pharmaceutical, Inc., Plainview, New Jersey) was selected because its half-life has been estimated to be between 2 and 15 minutes (*3*). In addition, no intracellular or extracellular metabolite of NMU has been shown to have the carcinogenic property generally attributed to this drug (*3, 4*). The area selected for prospective tumor formation was the right buccal mucosa. The stimulus used to induce long-term cell proliferation was a 0.025-cm stainless steel wire that was ligated around the upper right second molar and projected into the buccal mucosa. The wire served as a continuous, nonspecific, mechanical irritant which resulted in a sustained, local proliferative reaction. It has been shown that this kind of irritation results in hyperplasia, which, however, does not lead to tumor formation (*5*). We assessed the tumor incidence and latency by varying the time at which the NMU injection was administered in relation to the initiation of the mechanical irritation. Therefore, NMU was injected 7 days after (group 1) as well as 7 days before the mechanical irritation was applied (group 2). The left buccal mucosa, where no stainless steel wire was placed, was considered a control for the targeted area of prospective tumor development in the same animal. Other controls included animals injected with NMU in which no wire was placed (group 3), animals in which the wire was placed but no carcinogen was injected (group 4), and animals that were neither injected with NMU nor irritated with the wire (group 5).

Table 1 shows the incidence of tumors in the five groups of rats. In group 1, the mean time for tumor production in the irritated area of the buccal mucosa calculated from the day of carcinogen injection to the day on which the animals were killed was 131 ± 20.69 days, with a median of 105 days. In group 2, the

Table 1. Location and incidence of soft tissue tumors in experimental and control animals. The numbers in parentheses indicate the number of animals in each group. All lesions of the oral cavity that are listed as tumors were histologically malignant (see Fig. 1).

| Location | Group 1 (18) | Group 2 (29) | Group 3 (30) | Group 4 (29) | Total* (116) |
|---|---|---|---|---|---|
| Buccal mucosa irritation site | 7† | 7‡ | | | 14 |
| Other sites of oral mucosa | | | 1§ | | 1 |
| Abdominal wall | 1 | 1 | | | 2 |
| Intestine | | 3 | 1 | | 4 |
| Kidney | 1 | 2 | 1 | | 4 |
| Lung | 1 | 2 | | | 3 |
| Vertebrate marrow | 1 | 1 | | | 2 |
| Other systemic locations | | 1 ‖ | 2¶ | | 3 |

*A group of ten male W/F rats not subjected to any treatment and kept for 11 months (group 5) is included in the total. No tumors developed in this group. †Five spindle cell tumors, one undifferentiated sarcoma, one fibrosarcoma. ‡Four spindle cell tumors, two undifferentiated sarcomas, one round cell carcinoma. §Spindle cell tumor between the lower incisors. ‖ Skin tumor. ¶Spleen and skin tumors.


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