examined, increases in the density of receptors do occur as a consequence of denervation. This has been well characterized with nicotinic cholinergic receptors on skeletal muscle (*5*) and has also been observed with α-adrenergic (*6*) and dopaminergic (*7*) receptors as well as with β-adrenergic receptors. On the other hand, denervation causes other changes in addition to altering receptor density (*8*). For example, in smooth muscle changes in membrane potential appear to account for a relatively nonselective increase in neuronal sensitivity (*9*).

We feel that a more likely explanation for the lack of effect on β_2 receptors in the cerebral cortex is that the input to these receptors is not neuronal. In this case, alterations in neuronal firing would have no effect on these receptors. We previously showed that although the density of β_1-adrenergic receptors in discrete areas of rat brain varies almost 20-fold, the density of β_2 receptors varies only two- to threefold, suggesting that β_2 receptors are located on a more homogeneously distributed tissue constituent than are β_1 receptors (*10*). It should be noted that β_2 receptors have a low affinity for norepinephrine (the major adrenergic catecholamine in the central nervous system). One reasonable hypothesis is that β_2 receptors in the cerebral cortex are associated with blood vessels and that their endogenous input is epinephrine released from the adrenal.

In summary, the observation that the densities of β_1- and β_2-adrenergic receptors in the brain can be independently regulated provides strong evidence that they are indeed separate receptors, that they probably exist on distinct tissue components, and that they receive different endogenous inputs.

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Induction of Differentiation in Human Promyelocytic Leukemia Cells by Tumor Promoters

Abstract. *Phorbol diester tumor promoters and the promoter mezerein convert human promyelocytic leukemia cells in culture into adherent, nonproliferating cells with many of the characteristics of macrophages. Other types of promoters such as anthralin, phenobarbital, and saccharin do not have this effect. Various compounds that can inhibit some of the biological and biochemical effects of tumor promoters do not interfere with the induction of cell adherence and differentiation by the effective promoters.*

Tumor promoters are compounds that enhance tumor formation when administered after a low dose of a carcinogen (*1*). Phorbol diesters and some related plant diterpene esters that promote tumors in mouse skin in vivo have been shown to exert many biochemical and biological effects on cells in culture (*2*, *3*). Recently, it has been reported (*4*-*7*) that these compounds can reversibly inhibit terminal differentiation of various normal and malignant cells in culture. For example, they inhibit spontaneous and induced differentiation of Friend erythroleukemia cells (*4*, *6*). A possible relationship between this effect of promoters in vitro and the mechanism of tumor promotion in vivo has been discussed by Weinstein and Wigler (*8*) and Diamond *et al.* (*3*).

During an investigation of the generality of promoter-mediated inhibition of differentiation, we observed that these compounds can have more than one effect on differentiation, the effect depending on the target cell. While this work was in progress, Miao *et al.* (*9*) reported that in two Rauscher virus-transformed murine erythroid cell lines phorbol diester tumor promoters stimulated, rather than inhibited, differentiation along the normal erythroid pathway. We now report that in a human promyelocytic leukemia cell line, HL-60 (*10*), some tumor promoters can induce differentiation along a pathway apparently different from that normally followed. Thus, tumor promoters can have at least three different effects on terminal cell differentiation: inhibition of differentiation, stimulation of the normal pathway, and, as reported here, induction of an alternate pathway.

The HL-60 cell line was derived from the peripheral blood leukocytes of an adult human female with acute promyelocytic leukemia (*10*). Most of the cells are promyelocytes, but approximately 20 percent of the population consists of more mature myeloid cells. As with Friend murine erythroleukemia cells (*11*), HL-60 cells can be induced to differentiate by treatment with dimethyl sulfoxide, butyric acid, or dimethylformamide (*12*). Differentiation of HL-60 cells then occurs along the myeloid series and metamyelocytes, banded granulocytes, and mature granulocytes are formed.

When HL-60 cells were treated with the potent tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), they became adherent to the plastic substrate and gradually underwent morphological and biochemical changes indicative of differentiation along the monocytic rather than the normal myeloid series (Fig. 1). The cell shape changed dramatically, the azurophilic granulation typical of promyelocytes disappeared, and, after 48 hours, the nuclear : cytoplasmic ratio was shifted from greater than 1 to less than 1 (see Fig. 1). At an initial cell density of 2×10^5 per milliliter and a TPA concentration of 1.6×10^{-8} to $1.6 \times 10^{-7} M$, 20 to 40 percent of the cells were attached to the plastic surface after 18 hours and 80 to 90 percent were attached after 48 hours. Some cells also adhered to each other, forming small clumps that attached to the surface. The small fraction of cells that did not become adherent nevertheless lost all proliferative potential; no proliferating cells were ever recovered after exposure of HL-60 cells to TPA concentrations of $10^{-8} M$ or greater. Autoradiography (*13*) revealed that the number of cells incorporating [^{3}H]thymidine over a 24-hour period decreased from 90 percent in the untreated suspension culture to 12 percent of the adherent cells at 24 hours after TPA treatment ($1.6 \times 10^{-7} M$) and 0 percent at 48 hours.

The TPA also induced many biochemi-

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cal and functional changes in HL-60 cells; these are described in detail in (*14*). Briefly, TPA-treated HL-60 cells differ from the control cell population in that they are able to phagocytize latex beads and sensitized red blood cells, the amount of lysozyme excreted into the culture medium is increased eightfold and the amount of intracellular acid phosphatase is increased tenfold, all cells have nonspecific esterase activity as demonstrated histochemically, and receptors for immunoglobulin G are present on the cell surface. Thus, with respect to these properties, cell morphology, and growth potential, TPA-treated HL-60 cells resemble macrophages and differ from myeloid precursors and granulocytes.

The ability of other tumor-promoting compounds (*1*, *15*) to induce adherence and differentiation of HL-60 cells was compared with that of TPA by determining the number of adherent cells 48 hours after the addition of the compounds to suspension cultures (Fig. 2). All the phorbol diester tumor promoters induced adherence of HL-60 cells. The most active were TPA, phorbol-12,13-didecanoate, and phorbol-12,13-dibenzoate; phorbol-12,13-diacetate had slight activity; and 4α-phorbol-12,13-didecanoate and the parent alcohol, phorbol, were inactive. The differences in the ability of these compounds to induce differentiation in this system are similar to the differences in their ability to inhibit differentiation in other cell systems (*4*-*6*). Of the other tumor-promoting compounds tested for inducing activity, only mezerein, another plant-derived diterpene ester, was active (Fig. 2). Anthralin ($10^{-6}M$), phenobarbital ($10^{-6}M$), and saccharin ($8 \times 10^{-3}M$) were all inactive.

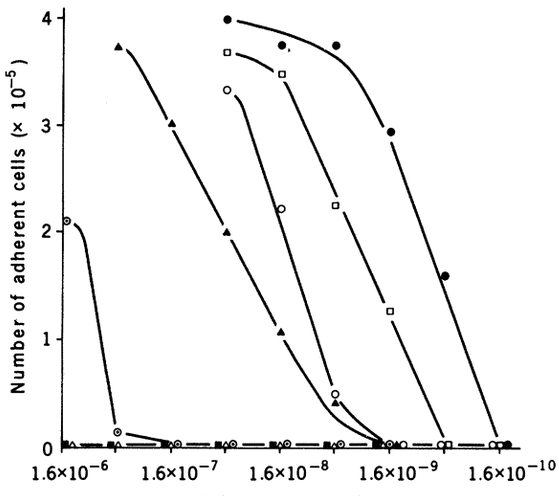

Fig. 2. Effect of tumor promoters on the adherence of HL-60 cells. Cells were seeded at a density of 0.5×10^5 per milliliter in 10 ml of medium in 25-cm² Falcon flasks. The various compounds were diluted in medium from ×1000 stock solutions in acetone and added to the cultures at the final concentrations indicated; control cultures were treated with 0.1 percent acetone. After 48 hours the adherent cells were detached by trypsinization and the number was determined in a Coulter electronic cell counter. Concentrations of each compound higher than those shown were toxic. Symbols: (●) TPA; (□) phorbol-12,13-didecanoate; (▲) phorbol-12,13-dibenzoate; (⊙) phorbol-12,13-diacetate; (△) 4α-phorbol-12,13-didecanoate; (■) phorbol; and (○) mezerein.

Several compounds that have been reported to inhibit one or more of the biological or biochemical effects of the phorbol diester tumor promoters (*16*, *17*) were tested for their effect on TPA-induced differentiation of HL-60 cells (Table 1). None of the compounds blocked the response of HL-60 cells to TPA, even when the cells were pretreated with a concentration of the test compound 1000 times greater than the concentration of TPA used to induce differentiation.

To our knowledge, this is the first report that phorbol diester tumor promoters can not only inhibit differentiation and stimulate differentiation along the normal pathway but can also induce differentiation along an alternate pathway. This suggests that a major determinant of the response to these compounds may be the target cell—for example, whether it is a unipotent or multipotent stem cell. The finding that tumor promoters can have opposite effects on differentiation in different cell types does not rule out the possibility that the mechanism of tumor promotion involves some effect on normal cell differentiation (*3*). The specific effect that is relevant to promotion may be determined by the target tissue involved and may not be the same in all tissues.

The chemical structure of the promoters is also a determinant of cellular effects; only diterpene esters that are promoters in vivo induced adherence and differentiation of HL-60 cells. Yamasaki *et al.* (*18*) observed that these esters induce adherence in some clones of

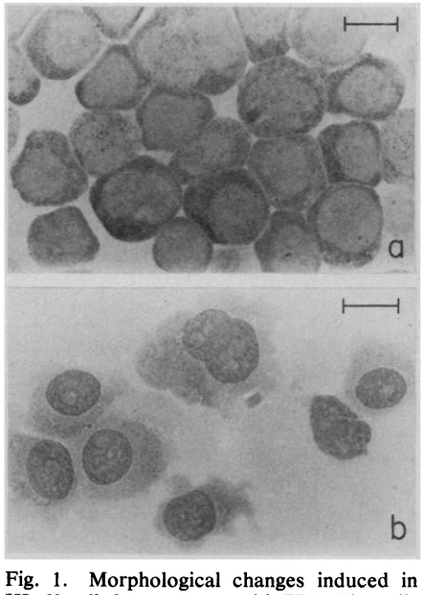

Fig. 1. Morphological changes induced in HL-60 cells by treatment with TPA. The cells were grown in suspension culture in RPMI 1640 medium supplemented with 15 percent fetal bovine serum. They were seeded into Falcon plastic flasks at a density of 2×10^5 per milliliter and TPA was added at a final concentration of $1.6 \times 10^{-8}M$. Slides of the control cells in suspension and of the treated adherent cells, detached by trypsinization, were prepared by using a Shandon-Elliott cytospin centrifuge. (a) Untreated cells; (b) cells treated with TPA for 2 days (May-Grünwald Giemsa stain; scale bars, 20 μm).

Table 1. Effect of compounds with antitumor-promoter activity on TPA-induced adherence of HL-60 cells. The HL-60 cells were seeded at a density of 1×10^5 in 10 ml of medium in 25-cm² Falcon flasks and treated with the antitumor-promoting compounds (*16*). The TPA was added to the cultures either simultaneously or 24 hours later at a final concentration of $1.6 \times 10^{-9}M$. The number of adherent cells was determined 48 hours after the addition of TPA. Cultures pretreated with the test compounds for 24 hours showed no adherent cells before addition of TPA. The increased number of adherent cells in these cultures compared to the number in cultures treated simultaneously with TPA reflects the cell replication that occurred before TPA was added. Concentrations were $10^{-3}M$ for antipain and leupeptin and $10^{-6}M$ for all other test compounds. Results shown are averages for duplicate cultures.

Test compound	Total number of adherent cells ($\times 10^{-5}$)	
	Simultaneous treatment	Pretreatment
TPA alone	5.9	9.9
TPA + fluocinolone acetonide	8.2	12.8
TPA + retinoic acid	6.8	11.6
TPA + dexamethasone	5.2	12.4
TPA + antipain	4.9	10.9
TPA + leupeptin	5.3	10.6
TPA + indomethacin	6.4	10.1
No TPA	0	0

Friend erythroleukemia cells. Adherence and differentiation of HL-60 cells were not induced by promoters, such as anthralin, that are unrelated in chemical structure to the plant diterpenes and are much less active in vivo. A similar difference between the effects of phorbol diester tumor promoters and other types of tumor promoters on cells in culture has been observed by others. Driedger and Blumberg (*19*) found that TPA and nonphorbol tumor promoters had different effects on the large-external-transformation-sensitive (LETS) glycoprotein and on deoxyglucose transport in chick embryo fibroblasts. Wigler *et al.* (*20*) found in the same cells that only diterpene esters, and not other types of tumor promoters, induced plasminogen activator. It is possible, therefore, that many of the effects of tumor promoters on cells in vitro are unrelated to the mechanism of promotion or that different chemical classes of promoters have completely different mechanisms of promoting action. Driedger and Blumberg (*19*) reached a similar conclusion. It is also of interest in this connection that a compound such as fluocinolone acetonide, which can inhibit TPA-induced tumor promotion in vivo (*16*) and TPA-induced DNA synthesis in mouse epidermal cells in vitro (*21*), had no effect on the induction of differentiation by TPA in HL-60 cells.

It is also interesting that mezerein, one of the plant esters that was an effective inducer of differentiation in the HL-60 cell line, has been shown to exert antileukemic activity against the P388 murine lymphocytic leukemia (*22*). Its ability to force proliferating human promyelocytic leukemia cells to differentiate into nonproliferating macrophage-like cells suggests that it and similar compounds may merit further investigation as antitumor drugs with this particular mechanism of action. It should be stressed that the loss of proliferative capacity induced in HL-60 cells by mezerein and the phorbol diester tumor promoters is not only very rapid but also affects 100 percent of the cell population. In contrast to the effects of other types of inducers of differentiation such as dimethyl sulfoxide in this and other cell systems, there are no nonresponsive, proliferating cells remaining in HL-60 cell cultures treated with effective tumor promoters.

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Species-Specific Perceptual Processing of Vocal Sounds by Monkeys

Abstract. *Monkeys of four species were trained to discriminate between sets of natural tonal calls of Japanese macaques (*Macaca fuscata*) by the position of a frequency-inflection peak or by initial pitch. The Japanese macaques consistently performed best on peak position and the other species on pitch. The results imply special strategies for perceptual processing of vocal sounds and suggest parallels with human speech perception.*

In recent studies of the psychology and physiology of hearing, there is evidence of novel insights from the use of complex, biologically significant stimuli (*1*). Investigators are increasingly concerned that organisms may use special perceptual procedures in the processing of sounds with particular significance to species members, such as those used in social communication. The evolution of speech has evidently capitalized on particular attributes of the human auditory system, although the extent to which these are uniquely human is moot (*2*). We have undertaken to analyze how Japanese macaques (*Macaca fuscata*) perceive elements from their repertoire of communicative sounds to determine whether they have evolved predispositions for processing such signals. The aim is to discover basic commonalities and contrasts between speech perception and the perception of conspecific calls by monkeys. An earlier report described our discovery that Japanese macaques consistently exhibit right-ear dominance while engaged in the perceptual processing of these vocalizations (*3*). Here we explore whether these monkeys use other strategies characteristic of speech perception in the processing of their own vocalizations.

One task a human listener faces when decoding speech sounds is the extraction of the appropriate linguistic content in the face of variability introduced by differences in the speaker's age, sex, and individual vocal tract anatomy. This normalization process has been termed perceptual constancy (*4*). Recent data in-

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