Glucocorticoid Receptors in Lymphoid Tumors

Brigid G. Leventhal
Division of Pediatric Oncology, Johns Hopkins Hospital, Baltimore, Maryland 21205

Abstract

There is a range of levels of glucocorticoid receptor numbers seen in the various subclasses of acute lymphatic leukemia (ALL). This variability cannot be explained by the known correlation between active cell proliferation and an increase in the number of receptors, since the tumors with the highest growth fraction (i.e., Burkitt's lymphoma and T-cell leukemia) tend to have lower average receptor numbers than do tumors with lower growth fractions such as common ALL. All clinical specimens from patients with lymphatic leukemia have some measurable level of glucocorticoid receptors; therefore, the resistance seen in vivo cannot be explained by the lack of receptors. However, there has been a positive correlation, in our hands, with receptor level and prognosis. On the basis of in vitro models, it is proposed that perhaps the high receptor cell lines (i.e., common ALL of childhood) have relative stability of their genetic material making glucocorticoid-resistant mutations less likely to occur in patients with these cells than in low-receptor cell lines (i.e., T-cell leukemia). This greater genetic variability in the low-receptor lines could account for the earlier emergence of clinical glucocorticoid resistance in these patients.

In considering the significance of cellular glucocorticoid receptors, it is important to enumerate at least some of the host or environmental factors which may alter receptor level before considering the cell itself. First, one must consider the species in which the cell arises. Steroids exert markedly different effects in various species. Claman (3) has classified the mouse, rat, and rabbit as steroid-sensitive species on the basis of the relative susceptibility of their lymphocytes to lysis by steroids, while lymphocytes from other species (guinea pigs and humans) are resistant. Thus interspecies comparisons must be made with caution. The hormonal state of the individual may also influence receptor level. Duval and Homo (5) have demonstrated, for example, that adrenalectomy in mice can produce not only an increase in thymus size and cellularity but also an augmentation of the number of dexamethasone-binding sites per cell. Others have shown a fall in total receptor number after adrenocorticotrophic hormone treatment or adrenal ablation. The existence of such a mechanism of receptor regulation emphasizes the idea that receptor modulation will occur under conditions when fluctuations of steroid plasma levels are observed such as circadian rhythms, stress, and steroid therapy. In addition, in humans, Fauci and Dale (6) have demonstrated that steroid treatment induces changes in lymphocyte recirculation and homing, so that the apparent lymphopenia after glucocorticoid administration to normal volunteers is due to movement of lymphocytes out of the circulation rather than lympholysis. The age of the individual may affect the level of steroid receptor; Roth and Livingston (13) have reported a decrease in receptor content in leukocytes with senescence.

The extent of steroid binding to the receptors is also dependent on other modulating factors such as metabolic conditions. For example, Munck et al. (11) have shown that the binding of cortisol to its cytosolic receptors was virtually abolished in the absence of glucose or under anaerobic conditions.

Once one has considered the milieu in which the cell exists, one must also consider the cell itself. In steroid-sensitive species, the most immature T-cells are also the most steroid sensitive. With maturation or with exposure to specific antigen, relative steroid resistance is acquired (8). Although receptors have not been measured in these circumstances, it is probable that there are changes. In a given cell, also, the level of binding sites fluctuates according to the stage of cell proliferation. Cidlowski and Michaels (2) demonstrated in synchronized HeLa cells that the number of glucocorticoid receptors increases during S phase and falls after mitosis. Blast transformation in response to phytohemagglutinin (12) and concanavalin A (14) results in increased levels of glucocorticoid receptors. Smith et al. (14) believe that this is related to the stage of the cell in cycle. They have demonstrated in their concanavalin A-stimulated cells that, with increasing time after concanavalin A stimulation, there is an increase in number of receptors and an increase in the amount of tritiated thymidine incorporated into the cells; however, the percentage of inhibition of thymidine incorporation in the presence of dexamethasone remains stable.

In studying normal human cells, Lippman and Barr (9) used rosetting techniques to study purified populations of T-, non-T, and mononuclear cells from human peripheral blood and discovered that there is no difference between T- and non-T lymphocytes in receptor content on a per cell basis or in affinity for glucocorticoid, with each lymphocyte population containing about 3000 sites/cell. Monocytes under these same conditions contain about 7000 sites/cell.

Yarbro et al. (15) attempted to correlate leukemic cell type in humans with glucocorticoid receptor level. These studies were all done with a whole-cell method in which the leukemic cells were incubated with varying concentrations of labeled dexamethasone which was then chased with cold dexamethasone to assure specificity of binding. In this study, T-lymphoblasts (defined by the erythrocyte-rosetting technique) had significantly lower levels of glucocorticoid receptors than did null lymphoblasts. The binding curves for each cell type showed that, although the total number of sites per cell differed markedly, the shape of the curves was the same, with both approximately 70% saturated at a concentration of 0.8 × 10^-8 M dexamethasone. The dissociation constants were also about the same, with Kd about 3.9 × 10^-8 M for both cell types. This difference in receptor number cannot be explained on the basis of cell cycle events since the cells with the higher thymidine labeling index (T-cells) had the lower receptor level.

The null group of cells can be further subdivided into those cells which did stimulate allogeneic donors in mixed-leukocyte

1 Presented at the Conference on Cell Markers in Acute Leukemia, March 4 and 5, 1980, Bethesda, Md. Supported in part by Grant CA 28476.
Thus, the presence of a receptor is required for steroid effect. From studies with these lines, one can conclude that glucocorticoid receptors. There are, in addition, some mutants, to the effects of glucocorticoids. The most common mutation (7) are grown in varying concentrations of glucocorticoid in the strong correlation with remission duration.

T-cell categories, and 5 of 18 were still in remission with a to the following hypothesis. While 2 copies of a gene (r+,r+) frequency. The S49 line has about 14,000 receptor sites per which glucocorticoid-resistant variants arise only at very low frequency, and the other, the W7 murine thymoma line, in ant to glucocorticoid-induced killing arise at a surprisingly high frequency. She studied 2 cell lines; one, the S49 murine lymphoma, in which they compared null cell leukemic cells with pre-B leukemic cells. In their 10 pre-B patients, they found higher levels of glucocorticoid receptors (21,000 sites per cell) than in their 14 null cell patients who had a mean of 14,000 sites per cell. The 9 T-cell patients had 2,500 sites per cell. These results are not yet statistically significantly different; however, it seems probable that subcategories of leukemic cells within the non-T group may differ in glucocorticoid receptor level as well as in other properties.

In a subsequent analysis, Lippman et al. (10) examined the effect of receptor level on initial complete remission induction and duration. Cells from a total of 45 patients were studied. Of patients with low levels of receptor (less than 2500 sites per cell), all had T-lymphoblasts; none remained in remission at the time of the report, and the median remission duration was 7.6 months. All patients with receptor levels over 6000 sites per cell had null cell disease. Of these, 10 of 16 remained in complete remission, and the median remission duration was over 31 months. Patients with intermediate levels (over 2500 and less than 6000 sites per cell) were in both the null and the T-cell categories, and 5 of 18 were still in remission with a median remission duration of about 20 months. These levels are significantly different from each other at p < 0.001. Thus, independent of cell type, glucocorticoid receptor level was strongly correlated with remission duration.

If lymphoid tissue culture cell lines from mouse (1) or humans (7) are grown in varying concentrations of glucocorticoid in the presence of mutagens, clones of cells arise which are resistant to the effects of glucocorticoids. The most common mutation which occurs in these cell lines is the absence of cytoplasmic glucocorticoid receptors. There are, in addition, some mutants, which have either a low number or an abnormal form of nuclear acceptor sites. From studies with these lines, one can conclude that steroids at least at physiological concentrations will not affect the growth of cells which lack receptors completely. Thus, the presence of a receptor is required for steroid effect.

Bourgeois (1) has described an interesting in vitro situation which might have relevance to the clinic. She studied 2 cell lines; one, the S49 murine lymphoma, in which variants resistant to glucocorticoid-induced killing arise at a surprisingly high frequency, and the other, the W7 murine thymoma line, in which glucocorticoid-resistant variants arise only at very low frequency. The S49 line has about 14,000 receptor sites per cell, while W7 contains about twice that amount of receptor or 29,000 sites per cell. The receptors of both cell lines have the same affinity for dexamethasone (Kd = 1.3 × 10^-9 M). The presence of twice as much receptor in the stable W7 line led to the following hypothesis. While 2 copies of a gene (r+,r+) coding for the glucocorticoid receptor would be present in the W7 line, the S49 line would be functionally hemizygous for that locus (r+,r-). In the latter case, a single genetic event is required to inactivate the r+ allele and to produce the receptor-negative (r-,r-) mutant, while 2 events would be necessary to inactivate both copies of the r+ in the W7 line. If similar selection processes were operating on cell lines in vivo, then one would predict that the cell lines or tumors with the lower glucocorticoid number would be initially sensitive to glucocorticoid, but would be more likely to give rise to a resistant variant which might recur and kill the patient than would the high receptor line. Thus, the correlation of good prognosis with high receptor number would result not so much from the initial sensitivity to the effect of steroids but rather from the lowered chance that resistant variants would be selected during therapy in the high receptor line. This hypothesis could be tested by measuring large numbers of patients in each category at diagnosis and at subsequent relapse. If it were correct, the number of receptor negative patients at relapse should be higher in the group which starts with low receptor numbers.

References

Glucocorticoid Receptors in Lymphoid Tumors

Brigid G. Leventhal


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/11_Part_2/4861

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.