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Characterization of Glucocorticoid Receptors in Animal Lymphoblastic Disease: Correlation With Response to Single-Agent Glucocorticoid Treatment

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The clinical significance of initial DEAE chromatography of glucocorticoid binders in lymphoblastic disease was evaluated in an animal model. Domestic cats and dogs with lymphoblastic disease were treated with prednisone, 2 mg/kg/day, for 14 days, and the outcome of therapy was correlated with DEAE chromatograms of glucocorticoid binders, using ^{3}H-triamcinolone as ligand. Six of 30 animals had a single-peak low-salt binder species, similar to that seen in a subset of human leukemia, and none of these responded. Of the 29 animals with chromatograms identical to normal tissues, 6 had a complete response and another 11 a partial response. This distribution of responders is statistically significant (p = 0.02). Thus, the leukemia-associated single-peak DEAE species appears to be associated with glucocorticoid resistance, as defined by clinical responsiveness. In contrast, the two-peak normal pattern is a necessary, but insufficient, criterion for defining responsive disease.

UNLIKE THE SITUATION in breast cancer, where a clear and clinically useful relationship between estrogen receptor quantitation and the outcome of hormonal therapy exists,[1-3] the correlation between the presence and number of glucocorticoid binding molecules in leukemia cells and response to glucocorticoid therapy in these diseases is less well defined.[4-11] Direct studies of this relationship in humans are now prejudiced by the routine use of multiple agents in the treatment of the leukemias. Where single-agent glucocorticoid therapy has been used, there has been a trend toward high receptor numbers being associated with glucocorticoid responsiveness. The data of Mastrangelo et al. in acute lymphoblastic leukemia (ALL)[10] suggest that "low" receptor numbers, below an arbitrary limit of 4,000 sites/cell by their assay method, are associated with lack of response to single-agent glucocorticoids. However, "high" receptor numbers are not uniformly associated with responsive disease. Responders (n = 8) had a mean receptor number per cell of 6,600 ± 4,894 (range 4,134–17,684), whereas nonresponders (n = 11) had a mean receptor number of 6,344 ± 9,806 (range 0–20,791).

In studies employing multiagent treatment, such as the studies of Costlow et al.,[11] there appears to be a trend between receptor quantitation and clinical outcome both in terms of successful remission induction and length of remission, with higher receptor numbers correlating with the more favorable outcome. In addition, these studies suggest a relationship between receptor numbers and surface marker phenotype, with the more favorable common or "null" ALL having higher blast cell receptor numbers, a relationship first reported by Lippman et al.[9] It must be emphasized that the relationship being attempted is of an essentially nonphysiologic nature. Clinical outcome in the studies referenced is in response to multiple agent chemotherapy, not to single-agent glucocorticoids. This "marker" use of glucocorticoid receptors has not become established in clinical practice, in contrast to the extensive use of surface phenotyping and terminal deoxynucleotidyl transferase (TdT) determinations.

To reconcile the 60% response rate to single-agent steroids[12] with the presence of receptor material in the blast cells of virtually all cases of ALL, we have hypothesized that quantitative assays measure a heterogeneous group of binding macromolecules, of which only a subset function as physiologic mediators of glucocorticoid action.[13] We therefore initiated a series of studies comparing certain biochemical and biophysical characteristics of glucocorticoid binders in normal cells with those in leukemia cells to determine if abnormal, dysfunctional binding macromolecules exist in leukemic cytosols. Of these methods, ion-exchange chromatography, isokinetic and sedimentation analysis, and affinity for DNA have proven to be useful in analyzing receptor structure and function. By DEAE chromatography, labeled cytoplasmic glucocorticoid receptors from a variety of normal tissues can be resolved into two components.[14,15] These components, referred to as peak I (early eluting) and peak II (late eluting) are 3.5S and 8.5S, respectively. Peak I binds to DNA; peak II does not. Following activation (by heat, change in ionic strength, or pH), peak II com-

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plexes acquire peak I characteristics, changing from an 8.5S configuration to a 3.5S form, eluting from DEAE in the peak I area, and acquire affinity for DNA. The peak I material that is seen variably in the initial, "preactivated" DEAE chromatograms of normal tissues may be accounted for by slow activation occurring during the analytical procedures.[16] We have previously shown in human acute leukemia that a subset of cases have glucocorticoid binding macromolecules in the blast cells, with altered biochemical and biophysical characteristics.[13]

In 30 of 78 (38%) human leukemia patients studied by us, the unactivated DEAE chromatogram was "abnormal," with the presence of only a single DEAE species eluting in the peak I area, which was 2–2.5S and failed to bind to DNA with or without activation. We hypothesized that these leukemia-associated early eluting binders do not represent functional receptors and further speculated that this subset of cases would be clinically unresponsive to single-agent glucocorticoid therapy.[13] Because of the problems of treating patients with single-agent therapy, we elected to study the relationship between this 2–2.5S receptor and responsiveness to single-agent prednisone in an animal model using spontaneous lymphoblastic leukemia-lymphoma of domestic cats and dogs.[17] These diseases, which have been proposed as a model for lymphoblastic disease in humans, show a 40% response rate to single-agent glucocorticoid therapy.[18] In a preliminary survey, we established that the early eluting leukemia-associated glucocorticoid binder identified in human cases was also present in a subset of cases of animal lymphoblastic disease, occurring in the blast cells of 13 of 75 (17%) animals studied.

MATERIALS AND METHODS

Reagents

^{3}H-triamcinolone acetonide (^{3}H-TA) was purchased from Amersham, Arlington Heights, IL; Sephadex G-25 was obtained from Pharmacia Fine Chemicals, Piscataway, NJ; DEAE cellulose, DE-52, from Whatman, Kent, England; native calf thymus DNA cellulose from P.L. Biochemicals, Milwaukee, WI; activated charcoal (Norit A) from Fisher Scientific, Medford, MA; Biofluor from New England Nuclear, Boston, MA; and lymphocyte separation medium (LSM) from Bionetics, Kensington, MD.

Animals and Therapy

Domestic cats and dogs with histologically confirmed "lymphoblastic" disease, and with owners consenting to receptor studies on histologically involved tissues and treatment with single-agent therapy with prednisone, were eligible for the study. Treatment was with prednisone, 2 mg/kg/day for 14 days. Subsequent management was at the discretion of the treating veterinarian and the animal owner. Cells and tissues from 35 consecutive animals (3 cats and 32 dogs), which met these criteria, were obtained from the Angell Memorial Animal Hospital, Boston, MA. Details of the histology are shown in Table 1. All animals were newly diagnosed and none had received therapy prior to study. Blast cells were harvested from EDTA anticoagulated LSM-sedimented venous blood (3 animals), from histologically involved nodes (31 animals), or both (1 animal). Nodes and cell pellets (5×10^8–5×10^9 cells) were stored at −90°C until used.

Table 1. Details of Histology

	ALL	LbLD	ProLL	DHL	Total
Dogs	2	25	3	2	32
Cats	2	1	0	0	3
Total	4	26	3	2	35

ALL, acute lymphoblastic leukemia; LbLD, diffuse lymphoblastic lymphoma; ProLL, prolymphocytic lymphoma (diffuse); DHL, diffuse histiocytic lymphoma.

Criteria for Response

Complete response was defined as the disappearance of all assessable disease. Partial response was defined as 50% reduction in the products of the largest diameter and its perpendicular for all measurable lesions, with no appearance of any new lesions, and a 50% reduction in the circulating blast count. Animals that did not survive for a minimum of 3 days of therapy were excluded from the study (one exclusion). Assessment was at the end of the 14-day treatment period. The veterinarian was unaware of the results of the receptor study at the time of assessment.

Analytical Procedures

The cytosol preparation and labeling procedures used were modified from the broken cell labeling system of Sakaue and Thompson,[14] as previously described. The analytical procedures have been described in detail.[13,14] No quantitative assays were performed in this study.

RESULTS

In the study population, 6 of 35 animals (17%) had an abnormal single-peak DEAE chromatogram similar to that which we have identified in human leukemia.[14] Representative normal and abnormal chromatograms are shown in Fig. 1.

Of the 35 animals studied, 6 achieved a complete response and a further 11 a partial response. All 17 of the responders had normal receptor characteristics, as defined by DEAE chromatography, as did 12 of the 18 nonresponders. No animal with a single-peak DEAE chromatogram responded. These results are summarized in Table 2. The six leukemia-associated single DEAE peak binders were distributed in both species (1 cat, 5 dogs) and in all clinicopathologic subgroups, with the exception of ALL, where all 4 cases had normal binders. We did, however, encounter the single-peak pattern in ALL in the preliminary survey. Two of the 4 ALL cases, 13 of the 26 diffuse lymphoblastic lymphoma cases, 2 of the 3 prolymphocytic lymphoma cases, and none of the 2 diffuse histiocytic lymphoma cases responded to the 14 days of prednisone therapy.

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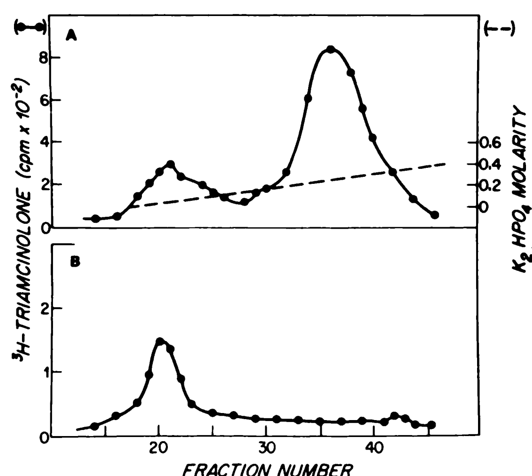

Fig. 1. DEAE chromatography of ^{3}H-TA binding macromolecules from blast cell cytosols. (A) A representative normal pattern chromatogram from a case of feline ALL. (B) A representative single 0.04 *M* salt peak chromatogram from a case of lymphoblastic lymphoma in a dog. Quantitative differences in cpm reflect grams of wet tissue in starting material.

DISCUSSION

The overall response rate to prednisone, 2 mg/kg for 14 days, was 17 (6 complete, 11 partial) of 35 (48%). This is similar to the response rate reported by Brick et al.[18] A single-peak (0.04 *M* salt) DEAE chromatogram of unactivated ^{3}H-TA-labeled glucocorticoid receptors would appear to be associated with failure of response to glucocorticoids in the animal lymphoblastic disease studied, with 0 of 6 animals responding. In contrast, a normal two-peak (0.04 *M* and 0.22 *M* salt) chromatogram is a necessary but insufficient criterion for response, with 17 of 29 (58%) animals responding. These differences are statistically significant at the $p = 0.02$ level. In human childhood ALL, the median time to response to single-agent glucocorticoids is 28 days.[12] In this study, we elected, for reasons of compliance, to limit the study to 14 days of therapy. The median time to response in these animals is not known, and it is possible that a longer exposure to glucocorticoids may have resulted in a higher response rate. In the context of this study, the initial DEAE chromatogram is clearly an inadequate criterion for selection of glucocorticoid-sensitive cases, with 42% cases with "normal" chromatograms not responding. This is similar to the 52% nonresponse rate for the whole group. It is likely that other defects in receptor physiology exist,

Table 2. Glucocorticoid Binders in Animal Lymphoblastic Disease

DEAE Pattern	Responders CR	Responders PR	Nonresponders	Total
Normal				
Peak I–peak II	6	11	12	29
Single peak (leukemia-associated 2.5S peak)	0	0	6	6
Total	6	11	18	35

Response versus DEAE pattern: $p = 0.02$ (Fisher's exact probability test).

and these are being sought in this subset of cases with normal initial chromatograms.

The purpose of this study was to attempt to assess the clinical and therapeutic significance of leukemic blast cell glucocorticoid receptor DEAE profiles. In ongoing work, we are assessing other biophysical parameters of the single-peak species (S value, DNA binding, isoelectric point) and studying the generation of this species. Our preliminary data suggest that this species resembles the proteolytically modified receptor species characterized by Sherman et al., Stephens and Stephens, and Wrange and Gustafsson.[19-21] It is possible that there is accelerated receptor degradation in these resistant cases, or alternatively, there could potentially be a mutation leading to an incomplete protein with an intact ligand binding site. Holbrook et al. have data consistent with accelerated receptor degradation in human leukemic blasts in vitro, with generation of meroreceptor.[22] Their mixing experiments support receptor "lability." However, the recent studies of Sherman et al.[23] do not show evidence of in vivo proteolysis of receptors in human leukemia cells and cell lines. Likewise, mixing experiments performed by us with human leukemia samples failed to suggest proteolysis as the basis for the single-peak binder.[13]

In one case of lymphoblastic lymphoma with the leukemia-associated single-peak binder, we fortuitously studied a noninvolved node. This had normal receptor characteristics on DEAE, suggesting that whatever the basis for the abnormality is, it is confined to the malignant clone. We are now studying involved and noninvolved tissues from animals that are "put to sleep" at the time of diagnosis to clarify this issue. We are at present searching for a stable source (cell line) of lymphoid cells with this abnormal binder, in order to fully characterize this species and to study the mechanism of its generation.

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