DETECTION OF SUSCEPTIBILITY TO CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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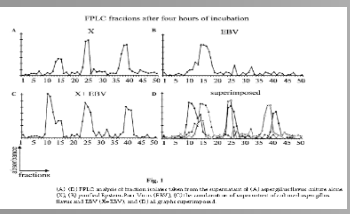
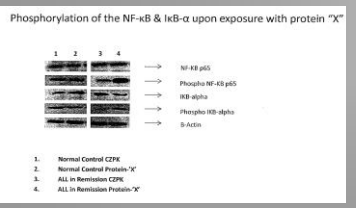
Introduction

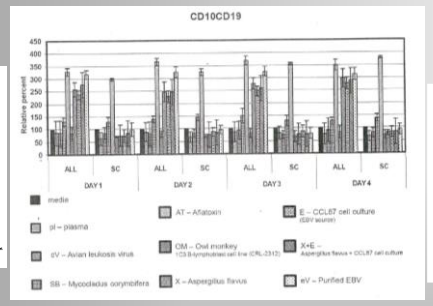
Currently, there are no known means to predict susceptibility to, and prevent ALL. We have separated, evaluated and patented a group of proteins dubbed 'Protein X' from a certain strain of Aspergillus Flavus (AF) and developed methods for screening, and identifying patients in remission and long term survivors of ALL, distinguishing them from "normal" controls. The 'Protein 'X' can reduce cell surface markers and upregulates NF-κB in ALL and has potential for screening and immunitaizon against ALL.

Materials and Methods

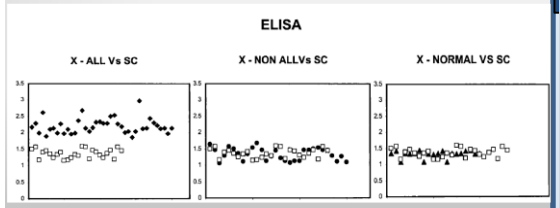
Mononuclear leukocytes (MNL) of ALL patients in remission and controls were separated using Ficoll Hypaque. Controls were normals, sickle cell and patients with tumors. Epstein Barr virus (EBV) was obtained commercially. Materials for control were aflatoxin, Mycocladus Corymbifera (MC), avian leukosis virus. Pre/post exposure MNL were co-incubated with 'Protein X' ± EBV ± irradiation, for periods of 1-72 hours. Controls were treated identically with appropriate substitutions. MNL were examined for genetic markers, NF-κB, ALL cell surface markers (CSM) and microarray. Changes expressed as percentage of control. Using ELISA, plasmas were tested for antibodies against 'Protein X' ± EBV.







This graph shows an example of the relative percentage of cells stained for one of the leukemic (ALL) cell surface markers CD10/CD19 at days 1-4 of incubation. X is the supernatant of the *aspergillus flavus* fungal culture; E is the supernatant from EBV-infected CCL-87 culture; pl is human plasma; ev is Epstein-Barr-Virus used alone (2X10^6 PFU);cV is avian leucosis virus 2X10^6 PFU/ml; AT is aflatoxin; OM is owl monkey cell culture supernatant. Mononuclear cells from ALL patients in remission was obtained to perform 40 separate tests. MNL from sickle cell patients undergoing routine partial exchange transfusions (discarded blood) was used as controls. Cell surface phenotypes (CD 10/19, CD 34/19, CD34/CD117) were examined daily for four days using a flow cytometer (BD FACS Canto I, Becton, Dickinson & Co., Franklin Lakes, N.J.) Results of all cell surface markers were similar, thus the result of CD10/CD19 are shown. These results are expressed as a percent of control.



(A)-(C) are graphs showing ELISA detection indicating a difference in long-term survivor leukemia patients compared to non-leukemic samples. (A) The supernatant from *aspergillus flavus* fungal culture (X) was incubated with ALL leukemic patients' plasma as compared to SC ("normal" controls): (B) The supernatant from *aspergillus flavus* fungal culture (X) was incubated with non-ALL cancer patients (Solid tumors) as compared to SC ("normal" controls): (C) The supernatant from *aspergillus flavus* fungal culture (X) was incubated with normal human samples from normal blood donors (discarded blood from blood bank), and compared to sickle cell patients' (SC) plasma.

Results

Upon 1-72 hours exposure of MNL from ALL patients in remission to 'Protein X' ± EBV, these developed cell surface phenotypes typical of ALL. Addition of EBV ± radiation to 'Protein X', enhanced these effects in MNL of ALL and not controls. These changes occurred with 3 major peaks of Aspergillus Flavus and were enhanced with addition of EBV and radiation. Changes were statistically significant $P < 0.001$ and separated ALL from controls. NF-κB revealed upregulation with 'Protein 'X' in ALL and not controls. Aflatoxin indiscriminately induced changes in cell surface phenotypes of both normal and ALL while MC had no effect. ELISA, using 'Protein X' ± EBV, distinguished ALL from controls. Micro array and biomarkers confirmed transformation to leukemic markers with exposure to 'Protein X' in MNL cells from ALL but not controls.

Conclusions

These studies reveal, in vitro, upon exposure to 'Protein X', unlike controls, MNL from ALL patients in remission, develop cell surface and genetic markers typical of ALL and up regulation of NF-κB. These techniques have potential for screening for ALL and may have implications for its etiology and prevention.

References

(1) Hendriks RW, Yuvaraj S, Kil LP; Targeting Bruton's tyrosine kinase in B cell malignancies. Nat Rev Cancer. 2014 Apr.14:210-232.
(2) Staudt LM; Oncogenic activation of NF-kappaB. Cold Spring Harb Perspect Biol. 2010 Jun;2(6):a000109.
(3) Thiel E; Cell surface markers in leukemia: biological and clinical correlations. Crit Rev Oncol Hematol. 1985;2(3):209-60.