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SIMULATION MODELING OF LYMPHOCYTE PROLIFERATION AND LYMPHOMA DEVELOPMENT IN IMMUNE COMPROMISED PATIENTS

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ABSTRACT

This paper examines the extent to which lymphoid cell proliferation contributes to the risk of malignant lymphoma in immunodeficiency patients. Recursive mathematical relationships encompassing mitotic rates, cell birth and death rates, and genetic transition probabilities are used in simulating the dynamics of the B cell population in the context of the two-event theory of carcinogenesis. The time-varying nature of cellular variables precludes the use of closed-form mathematical expressions in calculating cancer risk. Simulation results demonstrate that projected increases in lymphocyte proliferation brought on by immunosuppression and mitogenic stimulation are sufficient to account fully for the increase in lymphoma development among Burkitt's lymphoma patients, individuals infected with the human immunodeficiency virus, and organ transplant recipients.

1 INTRODUCTION

Cancer results from heritable changes in certain genes (Temin, 1988), as evidenced by the occurrence of mutational events within an undifferentiated lymphocyte population leading to non-Hodgkin's lymphoma (NHL) (Klein, 1979). In the presence of an underlying spontaneous risk of genetic error during mitotic activity, lymphoma incidence is enhanced by proliferative stimulation of target lymphoid cells, for example, by Epstein-Barr virus and holoendemic malaria in African children, by human immunodeficiency virus (HIV) in infected populations of various ages, and by various opportunistic infections in those immunosuppressed for organ transplantation (Purtilo, 1980; Beral *et al.*, 1991; List *et al.*, 1987). Using computer simulation of the lymphatic system B cell population, we analyze the relationship between increased cellular proliferation and the increased incidence of NHL in immunocompromised persons (Cohen and Ellwein, 1990a). Four immunodeficiency scenarios, endemic Burkitt's lymphoma (BL), neonates infected with HIV, adults infected with HIV, and organ transplant recipients are simulated and compared to a baseline scenario of non-endemic BL where immunodeficiency is less apparent.

2 BIOLOGICAL MODEL

Although a multitude of DNA-related changes may be observable in the development of malignant lymphoma, we assume that precisely two of these are necessary, and sufficient, once they become irreversibly fixed. We focus on the undifferentiated pro-B lymphocyte originating in the bone marrow as the target cell for NHL (Hansson *et al.*, 1983; Cooper, 1987; Nickerson *et al.*, 1989; Klein, 1989). The opportunities for the two critical genetic events are increased when immunosuppression and infection lead to an enhanced proliferation of the target cell population. Cellular dynamics are important because we assume that genetic changes take place only during the mitotic cycle, where each cell division is assumed to offer an opportunity for the required genetic rearrangements. A clinically recognizable lymphoma is present once malignantly transformed lymphocytes reach some threshold volume. This general interpretation of the carcinogenic process is illustrated in Figure 1 and provides the biological basis upon which the computer simulation model is constructed (Cohen and Ellwein, 1990a; Ellwein and Cohen, 1992).

As time advances each target cell is subject to one of the following events:

1) Cell death -- apoptosis of the undifferentiated target cell
2) Cell division producing two undifferentiated progeny

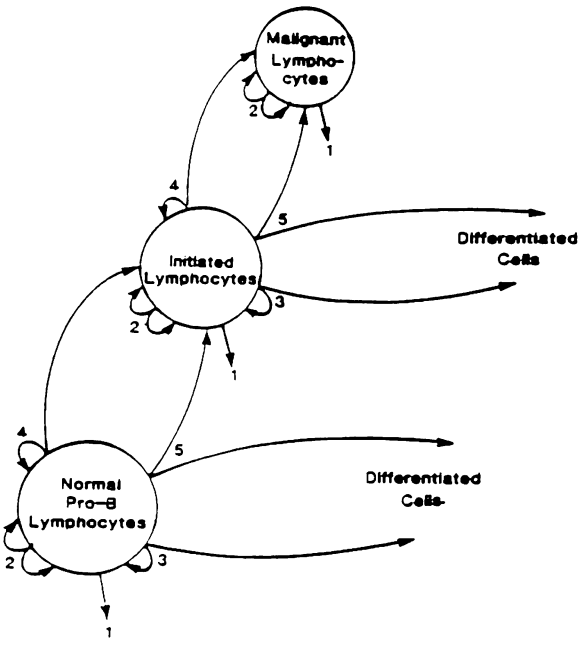

Figure 1: Proliferative and Genetic Transition Events Represented in the Simulation Model

3) Cell division with one undifferentiated replacement progeny and another destined for differentiation
4) Cell division with one replacement progeny and another with irreversible genetic change
5) Cell division with one progeny experiencing irreversible genetic change and the other destined for differentiation

Once a target pro-B cell becomes differentiated, it is no longer considered capable of the sustained replication necessary for lymphoma development. These cells are, therefore, not tracked within the model.

The rapid growth of the normal lymphoid cell population during the neonatal developmental period provides abundant opportunities for the genetic rearrangement required in creating an initiated cell. As initiated cells are generated, they, too, will multiply in number, providing opportunity for the second genetic change necessary for the cell to become fully malignant. Once a malignant cell is established, proliferation expands the pool to a clinically detectable tumor mass. Because genomic changes are assumed to be random phenomena, the size distribution of each of the three cellular states is defined probabilistically (Greenfield *et al.*, 1984).

3 SIMULATION PROCESS

The simulation begins with a cell population consisting entirely of normal undifferentiated pro-B lymphocytes, representative in number of some early time in the development of the lymphatic system. Advancing time is represented by a sequence of discrete time intervals. The subsequent distribution of cells across normal, initiated and malignant states changes in each time interval in response to model inputs specifying cell mitotic rates (M), cell birth and differentiation proportions (B), cell death rates (D), and conditional genetic transition probabilities (P). Recursive difference equations are used to update cell populations and other system variables with advancing time: The status of the organ system is expressed as a function of its status in the preceding time interval and values of input variables for the current time interval.

For example, the expected number of cells in state i at the end of the t^{th} time interval (E_i^t) is found using the following recursive relationship:

$$E_i^t = E_i^{t-1} - E_i^{t-1}D_i^t + E_i^{t-1}[M_i^t B_i^t(1 - P_i^t)(1 - D_i^t)]$$

$$- E_i^{t-1}[M_i^t P_i^t(1 - B_i^t)(1 - D_i^t)]$$

$$+ E^{t-1}_{i-1}[M^t_{i-1}P^t_{i-1}(1 - D^t_{i-1})].$$

Cells are added and subtracted from the cells present at the beginning of the i^{th} time interval, which is taken to be the number present at the end of the t-1 time interval (E_i^{t-1}).

In addition to the expected number of cells within each state, an output variable of primary interest is the probability that the number of malignant cells will exceed some clinically detectable number. The former value can be tested against empirical estimates of organ size or volume and the latter value against observed tumor incidence. These comparisons of simulation output with empirical data serve as the basis for model validation.

Except for mitotic rates, experimental data are generally not available for direct estimation of model inputs. Thus, the simulation exercise entails trial and error manipulation of model input variables until simulation outputs replicate empirical data.

The simulation exercise begins with calibration of the baseline model representing the B cell lymphoid population under normal conditions. Using empirically based estimates of mitotic rates, the first simulation runs focus on replicating development and matuation

Table 1: Estimates of Cellular Proliferation and Disease Prevalence

Scenario	Normal Cell Mitotic Rates	Normal Pro-B Cell Pool	Lymphoma Prevalence
Burkitt Lymphoma-sporatic (Baseline Scenario)	Daily rates: .05 at 1 year; .004 at 10 years; .0014 at 20 years	$.04 \times 10^{12}$ (steady state adult)	.00002 by 18 years with mode at 7-9 years
Burkitt's lymphoma-endemic	Enhanced 5-10X for 3-4 years starting at age 2 years	2 1/2X Baseline	.0015 by age 6 years with mode at 4-7 years
HIV AIDS - neonate	Continually enhanced 2X starting at age 1 year	2X Baseline	.03 by age 10 years
HIV AIDS - adult	Continually enhanced 20X starting at age 24 years	3X Baseline	.02 at 10 years post infection and .10 at 15 years
Solid Organ Transplant Adult	Enhanced 10-40X for 5 years after transplantation at age 34 years	1 1/2X Baseline	.005 at 6 years post transplantation

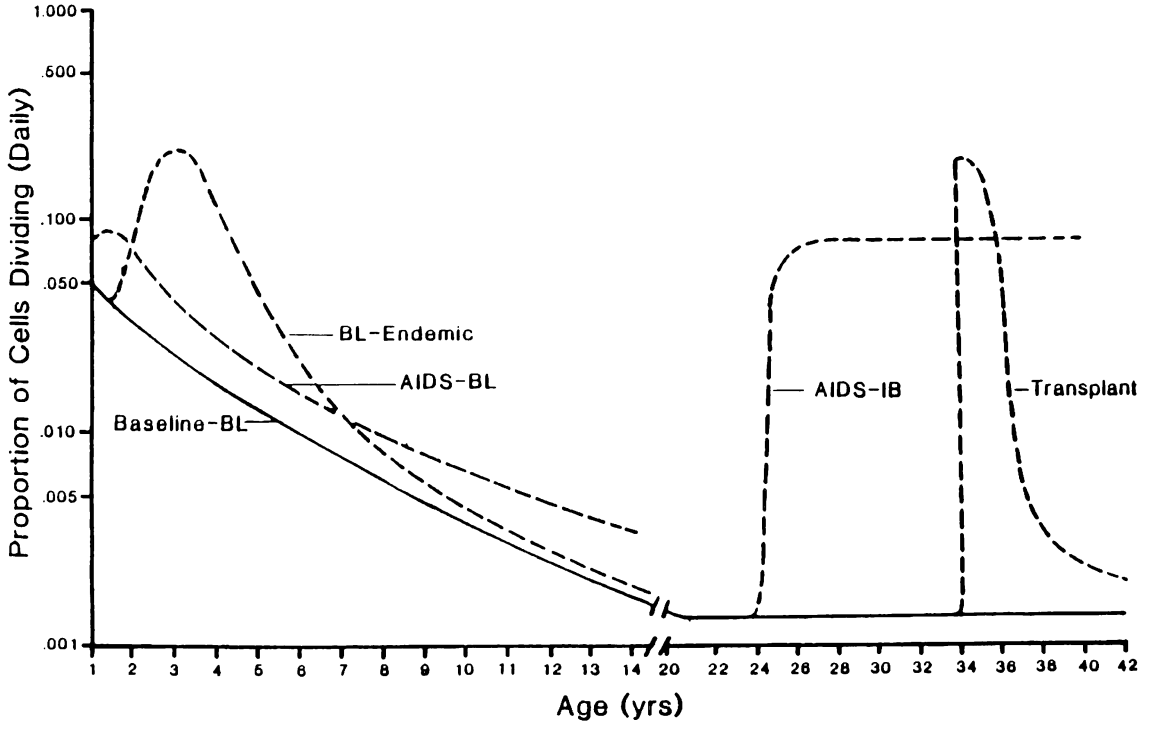

Figure 2: Mitotic Rates in Normal Pro-B Lymphocyte Populations

of the cell population without concern for reproducing spontaneous cancer induction. The genetic transition probabilities are set to zero and exploratory values for the time dependent birth/differentiation variable B_i' are used. The direct cell death variable D_i' is set to zero since significant cell death beyond the differentiation process is not common under normal conditions. Once actual lymphocyte population growth is simulated, replication of baseline lymphoma prevalence is addressed by upward adjustment of the genetic transition variables. This is attempted first with parameters for the initiated cell mitosis and birth/differentiation set to the corresponding values for the normal cell population, and then subsequently increased if necessary. Malignant cell mitotic rates are equated to that of initiated cells, but all cell divisions are assumed to produce two undifferentiated progeny (the birth/differentiation variable is set to 1.0).

Simulation of each of the four immunodeficiency scenarios proceeds by making adjustments to the baseline parameters. Cellular proliferation is simulated first, leaving conditional genetic transition probabilities at baseline levels. Once the postulated increase in the B cell pool is replicated, attention is given to increasing the transition probabilities from baseline levels, if necessary, until tumor prevalence is replicated. This multi-step iterative process has been described in detail elsewhere (Ellwein and Cohen, 1992).

4 MODEL PARAMETERS

Quantification of the target population of pro-B lymphocytes is based on data derived from mice, where after extrapolation to humans using body weight, we estimate a steady state level of 0.4×10^{12} pro-B lymphocytes by six years of age (Opstelten and Osmond, 1983). Beginning with daily cycling of these cells during the neonatal period, the mitotic rate drops, reaching an estimated daily mitotic rate of 0.05 by one year, 0.004 by ten years, with a leveling off at 0.0014 after 20 years. Based on information from clinical registries (Levin *et al.*, 1982; Burkitt and Wright, 1970), we estimate that the risk of developing non-endemic sporadic BL peaks around 7-10 years of age with a cumulative risk, or prevalence, of 2×10^{-5} by 18 years of age. It is assumed that the volume represented by 10^9 malignant B cells is sufficient for clinical detection of the disease.

In considering each of the immune deficiency scenarios, it is necessary to postulate the timing and extent to which cell proliferation is enhanced. Table 1 provides this information, expressed as increases in mitotic rates and the size of the total at-risk pro-B

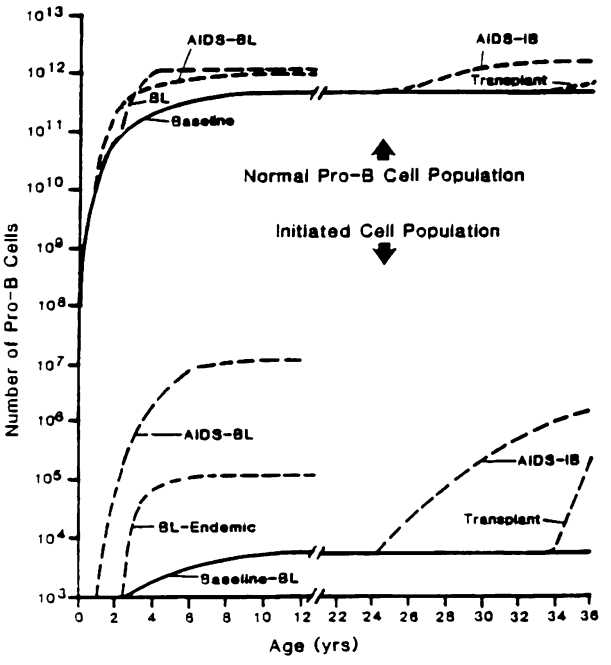

Figure 3: Model Estimated Normal and Initiated Lymphocyte Populations

lymphocyte pool compared to the baseline scenario. Essential to the hypothesis that increases in cellular proliferation alone can account for increases in disease prevalence is the ability to reproduce observed age-specific incidence of NHL represented by the four modeling scenarios (Beral *et al.*, 1991; Levin *et al.*, 1982; Burkitt and Wright, 1970; Poplack *et al.*, 1989; Patton *et al.*, 1990). To the extent that the resulting specification of cellular proliferation is sufficient in itself to account entirely for the increased lymphoma risk attributed to the experimental scenarios, it is not necessary to increase the probabilities associated with either the first or second genetic transitions above the 1.5×10^{-10} level established in the baseline analysis.

5 RESULTS

Figure 2 provides full detail on time-varying mitotic rates used in modeling the baseline case and the four experimental scenarios with enhanced cellular proliferation. The rates shown are for the normal pro-B cell population. Consistent with previous modeling analyses (Cohen and Ellwein, 1990b), a further 25-50% mitotic rate increase and a decreased likelihood of differentiation are used in representing the cellular dynamics of the initiated cell population. The resulting increase in both normal and initiated cell populations over baseline conditions is shown in Figure 3.

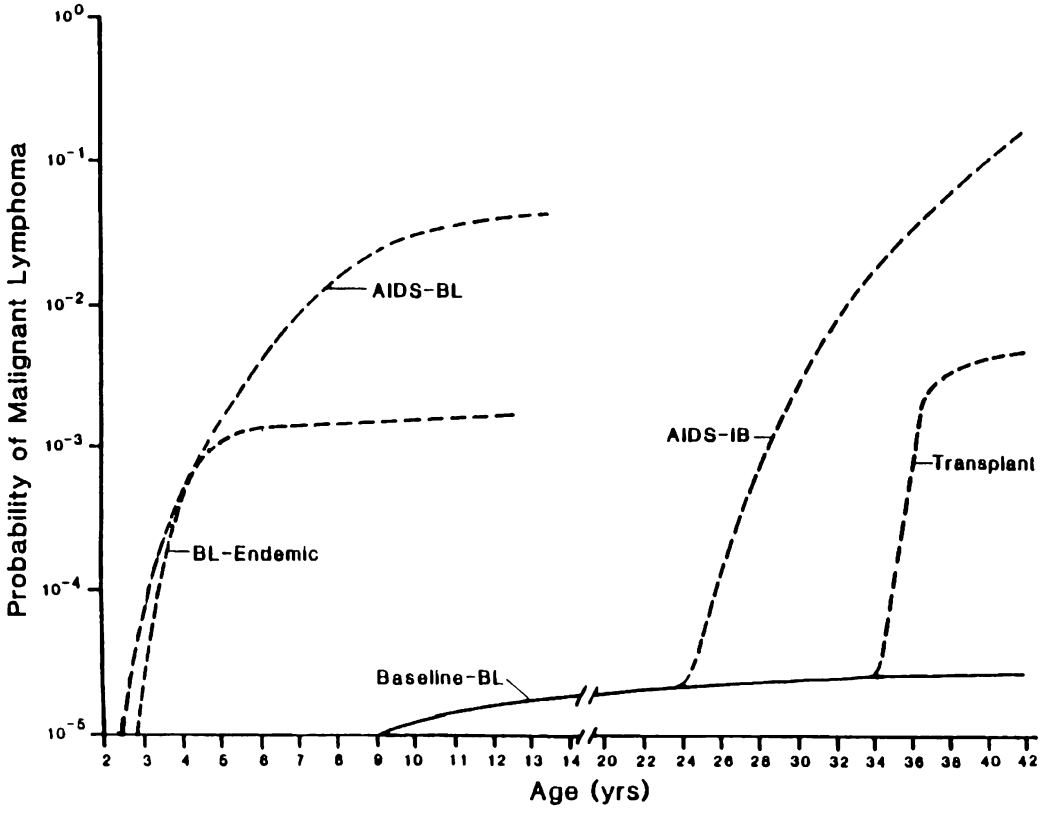

Figure 4: Cumulative Probability of Malignant Lymphoma for Baseline and Immune Deficient Scenarios

Figure 4 contains model projections of the cumulative probability of a clinically detectable malignant lymphoma as a function of patient age. The model does not account for the risk of death from other causes and, thus, the projections are those that would prevail if early death were not a significant factor. Also, since cellular proliferative responses were programmed to commence at a precise age for each of the scenarios, the curves of Figure 4 are more abrupt than they would be if a range of proliferation start-points were represented. Our modeling reflects the risk of lymphoma that might be faced by a "typical" individual within each of the experimental scenarios.

6 DISCUSSION

We were able to produce the entire set of simulation results without increasing either of the two conditional genetic transition probabilities from baseline levels. The increase in the initiated cell subpopulation (Figure 3) comes entirely from increasing over baseline the number of opportunities for spontaneous genetic error in normal cells -- through the sustained increase in normal B-lymphocyte mitotic rates and hyperplasia. Similarly, the enhanced dynamics of initiated cells ensures the relatively rapid appearance of one or more malignant cells, with proliferation to a clinically detectable mass. Thus, to the extent that the postulated increase in proliferation is plausible, these simulations demonstrate that cell proliferation alone can account for the increased tumor prevalence associated with the various immunosuppression scenarios: The mitogenic stimuli responsible for the enhanced cellular dynamics need not also directly damage DNA.

That cellular proliferation serves as a prelude to the emergence of a malignancy has been well established; however, only recently has biologically-based modeling of carcinogenesis incorporated quantitatively the effects of cellular expansion in understanding the genesis of malignancies (Cohen and Ellwein, 1990a; Greenfield *et al.*, 1984; Moolgavkar and Knudson, 1981). For example, the prevalence of experimental urinary bladder cancer predicted by computer modeling has

been validated by biological studies, wherein cellular mitotic events were quantitated with thymidine labelling and by morphometric accounting of the epithelial hyperplasia (Hasegawa *et al.*, 1986; Cohen and Ellwein, 1988; Ellwein and Cohen, 1988; Cohen and Ellwein, 1990b). We have recently reviewed the role of cellular proliferation in the genesis of a broad spectrum of malignancies in humans associated with viruses, chemicals and inherited predisposition (Cohen *et al.*, 1991).

The role of cellular proliferation in lymphomagenesis has been evaluated in quantitative terms by several groups of investigators during the past decade to arrive at hypotheses regarding the cellular proliferative events and specific cytogenetic errors that are likely to occur during lymphoma or leukemogenesis (Greaves, 1988; Murphy *et al.*, 1989; Morris, 1989; Okamota *et al.*, 1989; Cohen *et al.*, 1981; Altiok *et al.*, 1989). These investigations add to the recognition that increases in cell proliferation when coupled with an ever present background risk of spontaneous genetic error can be pivotal in explaining observed increases in tumor occurrence rates.

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