T Cell Function in Myelogenous Leukemia

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Introduction

There are several previous reports of proliferative reactions between remission lymphocytes and autologous or allogeneic cyopreserved blasts, in a proportion of patients with granulocytic and lymphocytic leukemias [1,2]. More recently, T lymphocytes cytotoxic for autologous leukemic blasts have been generated in vitro by a method involving allogeneic help [3–5]. These results suggest that leukemia specific antigens may exist on leukemic blast cells, and are immunogenic for autologous T lymphocytes.

If more detailed specificity studies confirm this interpretation, which would be compatible conceptually with the central dogma of tumour immunology derived from animal experiments, the immunotherapy of human leukemia with specifically immune autologous T cells becomes justifiable experimentally. For this approach to be practicable, large numbers of leukemia-specific T cells will be required.

T lymphocytes from normal donors have been maintained in culture for up to 13 months using a factor obtained from conditioned medium (LyCM) derived from phytohaemagglutinin (PHA) – stimulated normal human lymphocytes [6]. We have used this culture system to grow, selectively, T lymphocytes from the peripheral blood of patients with granulocytic leukemias in the untreated acute phase of the disease, and report here preliminary studies on the function of the cultured cells.

Methods

1. T Cell Culture

The procedure for T lymphocyte culture has been described previously [6,7]. Briefly, peripheral blood leukemic cells, obtained by leukophoresis, were seeded into 16 × 125 mm plastic tubes (Falcon Plastics, Oxnard, Calif.) at a concentration of $1-2 \times 10^5$ per ml of RPMI – 1640 medium supplemented with 20% heat inactivated fetal calf serum and 20% of four-fold concentrated LyCM (Associated Biomedic Systems, Buffalo, N.Y.). Cell concentration was maintained between 3×10^5 and 1×10^6 per ml by subculturing the cells in the same medium.

2. Mixed Leukocyte Culture

One way mixed leukocyte reactions (MLRs) were set up as described previously [7], using mitomycin-C treated autologous or allogeneic stimulating cells.

Results and Discussion

Fresh unfractionated peripheral blood white cells from only 11 of 26 patients with untreated acute phase myelogenous leukemias responded to allogeneic stimulation in the MLR.

Cells from a total of 15 patients were fractionated on nylon wool columns (Table 1). Alloresponsive cells were detected in either non-adherent or adherent populations in 3 out of 9 patients whose unfractionated cells were unresponsive. In another 3 patients, 8-03, 7-126 and 7-127, the magnitude of the allogeneic response of both non-adherent and adherent fractions was greater than that of the unfractionated population.

Table 1. Allogeneic and autologous responses of nylon fractionated leukaemic and normal peripheral blood white cells

Patient	Diagnosis	% Recovery		Allogeneic response			Autologous response
		Nylon non-adherent	Nylon adherent	Unfractionated	Nylon non-adherent	Nylon adherent	Non-adherent vs. Adherent
Normal		47,9	14,3	+	+ +	±	+
8- 03	CGL	7,6	24,7	+	+ +	+ +	+
7-126	AML	51,5	31,7	+	+ +	+ +	–
7-127	AML	46,1	19,0	+	+ +	+ +	–
7-105	AML	24,0	72,5	+	+ +	ND	–
7-119	AML	75,9	24,0	+	+ +	±	+
7-129	CGL	36,0	35,3	+	–	+	–
7-112	AML	50,0	26,7	–	+	–	+
7-133	AML	43,3	25,5	–	+	–	+
7-115	AML	14,7	19,0	–	–	+	–
7-107	AML	79,5	21,5	–	–	ND	+
7-113	CGL BC	34,0	37,3	–	–	–	–
7-117	CGL BC	64,0	23,3	–	–	–	–
7-110	AML	57,6	42,4	–	–	–	–
7-130	AML	54,7	17,3	–	–	–	–
7-131	AML	61,3	24,0	–	–	–	–

In 5 of the total of 15 patients whose cells were fractionated on nylon wool, non-adherent cells were stimulated by autologous nylon adherent cells. However, a similar autologous MLR was detected between the nylon wool fractions from all 10 normal donors tested.

In the residual 5 patients no allogeneic or autologous reactions were seen in the unfractionated or in either nylon wool fraction.

Samples from 13 patients were cultured in the presence of LyCM. Before culture, samples from the majority of patients with granulocytic leukemias had fewer than 5% E-rosette positive cells. In the presence of LyCM, the proportion of E-rosette positive cells after 12–17 days of culture is between 36 and 91%. After 26–36 days in culture, the percentage has increased to between 61 and 93%.

Cells cultured from all 13 donors showed significant alloresponses after culture (Table 2). Samples from 4 patients, whose fresh uncultured cells were unresponsive, all showed an allogeneic response after between 7 and 29 days in culture. The appearance of alloresponsive cells after culture in LyCM provides functional evidence that the E-rosette positive cells present after culture of leukemic peripheral blood are indeed T lymphocytes.

Cultured T cells from 8 patients were tested in a one way MLR for their response to autologous blast cells. In only 4 cases, however, was a significant response detected. Ia-like antigens have been detected serologically on a variety of leukemic blast cells [8], and Ia-bearing human B cells can stimulate histocompatible human T cells [9]. Evidence for in vivo immunization by leukemia-specific antigens therefore requires analysis of the specificity of the autologous proliferative reactions observed in vitro.

The present results indicate that T lymphocytes can be cultured from the peripheral blood of leukemic patients in the acute phase of the disease, which mount an allogeneic blastogenic response comparable to that of cultured T cells derived from normal donors. The appearance of such functional T cells

Table 2. Response of leukemia-derived cultured T lymphocytes to allogeneic stimulation in a one-way mixed lymphocyte reaction

Patient	Diagnosis	Days in culture	Stimulation index
7-11	AML	62	0,65
7-18	AML	35	5,92
7-22	Sezary syndrome	27	21,72
7-23	CGL	21	9,90
7-35	AMML	19	1,32
7-56	CGL	26	1,80
7-66	Acute undifferentiated leukemia	19	28,08
7-69	AML	12	156,41
7-82	ALL	11	10,80
7-92	AML	29	27,56
7-93	CGL blast crisis	29	13,89
7-97	AML	7	1,18
7-101	AML	21	41,34

after culture away from the leukemic microenvironment suggests that there is no functional defect in the residual circulating T cells in vivo. The autologous reactivity observed in some patients suggests that leukemic T cells may have the potential of becoming immunized by antigens on the leukemic blast cells. The availability of cultured T cells derived from leukemic donors offers the possibility of in vitro boosting of any such primary immunity, and autologous immunotherapy with in vitro boosted cultured T cells.

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