Archives of Disease in Childhood 1992; **67**: 524–526

T cell immunodeficiency in dyskeratosis congenita

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Abstract

Dyskeratosis congenita has been found to be associated with abnormal immune function. In this study we report a patient with this association. He developed *Pneumocystis carinii* interstitial pneumonia, and impaired cell mediated immunity was confirmed by the presence of depressed lymphoproliferative responses to in vitro stimulation with mitogen. Enumeration of T cell subsets showed a severely depressed CD4:CD8 ratio (0·38), which is the likely cause for impaired cell mediated immunity. The T cell activation pathway appeared intact, as his T lymphocytes were able to express activation markers (CD25 and HLA-DR) after mitogen stimulation.

Dyskeratosis congenita is a rare, heritable, multisystem disorder, characterised by reticulate skin hyperpigmentation, mucosal leucoplakia, and nail dystrophy. More serious features are bone marrow involvement with pancytopenia and a predisposition to malignancy. Although the primary defect has not been identified, an X linked mode of inheritance has been shown in the majority of cases.[1] Recently, linkage in one large family by using X chromosome specific RFLP markers has assigned the gene for dyskeratosis congenita to chromosome band Xq28.[2] Several families with apparent autosomal forms of dyskeratosis congenita have also been reported, suggesting aetiological heterogeneity in this disorder.[3 4]

Chromosome breakage is not a consistent finding, although enhanced G_2 chromatid radiosensitivity has been observed in the fibroblasts.[5] This phenomenon has been observed in individuals genetically predisposed to cancer,[6] a characteristic also observed in dyskeratosis congenita. Patients with this disorder are also prone to opportunistic infections, and immunological abnormalities which include both humoral and cell mediated dysfunction. We report a case of dyskeratosis congenita with abnormalities in T cell subsets and impaired lymphoproliferative responses to in vitro stimulation with mitogens.

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Accepted 18 December 1991

Case study

A 15 year old boy presented with characteristic dermatological features of dyskeratosis congenita which he had had since the age of 3 years. These included reticulate skin pigmentation, dystrophic nails, and oral mucosa leucoplakia. He had been immunised, including BCG, without complications. At the age of 10 years he had developed thrombocytopenia, which had progressed over the next few years to pancytopenia associated with bone marrow hypoplasia. At this stage a bone marrow transplantation was considered, but his parents refused consent. At the age of 15 he developed *Pneumocystis carinii* interstitial pneumonia, which was confirmed by the presence of protozoan cysts in the bronchial alveolar lavage. He responded to treatment with intravenous high dose co-trimoxazole. His male cousin (maternal side) was also similarly affected with dyskeratosis congenita, and died from sepsis at the age of 10 years.

His total white counts fluctuated from 6·4 to $0·8\times10^9$/l, with absolute lymphocyte counts of 2·5 to $<0·8\times10^9$/l. Serum immunoglobulin concentrations showed raised IgG 21·2 g/l, with normal concentrations of IgA 2·09 g/l, and IgM 2·1 g/l. HIV antibody was absent. Delayed hypersensitivity skin test to the purified protein derivative (10 unit dose) was negative, suggesting anergy.

Materials and methods

ISOLATION OF BLOOD MONONUCLEAR CELLS

Blood mononuclear cells (BMC) were prepared from heparinised blood by the standard Ficoll–Hypaque gradient.

CELL MARKERS

Cell surface expression of T cell subsets was assessed by indirect immunofluorescence using monoclonal antibodies against CD3, CD4, CD8 (Becton Dickinson). Half a million BMC were suspended in 100 µl Roswell Park Memorial Institute medium 1640 (RPMI-1640) supplemented with 2·5% fetal bovine serum and 0·01% sodium azide (staining buffer) and incubated with the respective monoclonal antibodies for 30 minutes on ice. This was followed by washing in staining buffer and a similar incubation with fluorescein isothiocyanate conjugated affinity purified goat antimouse immunoglobulin. The cells were then washed extensively and analysed using the FACScan flow cytometer (Becton Dickinson).

To assess the ability of T lymphocytes to express activation antigens upon stimulation with mitogen, cells were stimulated with phytohaemagglutinin 0·5 µg/ml. After a 48 hour incubation, the stimulated cells were stained separately with fluorescein-conjugated monoclonal antibodies against CD25 or HLA-DR, and phycoerythrin-conjugated CD3. The cells were read by two colour fluorescence analysis using the FACScan flow cytometer.

Table 1 Expression of the T activation markers CD25 and HLA-DR, shown by percentage increase in these factors

	Control		Patient	
	CD3 CD25	CD3 HLA-DR	CD3 CD25	CD3 HLA-DR
Medium	11	8	15	17
PHA 12·5 μg/ml	98	25	92	25

PHA, phytohaemagglutinin.

Table 2 Lymphoproliferative responses to mitogens

	^{3}H-thymidine incorporation (counts/min)			
	Expt 1		Expt 2	
	Control	Patient	Control	Patient
Medium	750	228	804	350
Phytohaemagglutinin	86 070	39 725	105 721	30 250
Concanavalin A	30 279	8 305	85 615	10 125

LYMPHOPROLIFERATIVE RESPONSES

Lymphocyte proliferative responses to the mitogens, phytohaemagglutinin (0·5 μg/ml) and concanavalin A (10 μg/ml) were assessed in microtitre plates containing 1×10^5 BMC suspended in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 100 U/ml penicillin and 50 μg streptomycin. Cells were pulsed with 1 μCi per well of tritiated thymidine (Amersham International) after 72 hours, and harvested 16–24 hours later using an automated cell harvester. Cell uptake of tritiated thymidine were then counted using a β scintillation counter (Beckman). Lymphocyte stimulation was assessed by the difference between the triplicate stimulated cultures and the unstimulated cultures measured in mean counts per minute.

Results

Evaluation of T cell numbers and subsets showed normal percentage of CD3 positive cells (76%). There was a reversal of CD4:CD8 ratio (which now measured 0·38), with percentages of CD4 and CD8 of 21% and 55%, respectively. The absolute numbers of CD4 positive cells were depressed ($0\cdot19\times10^9$/l; normal range $0\cdot25–1\cdot33\times10^9$/l), although the absolute numbers of CD8 positive cells were normal ($0\cdot50\times10^9$/l; normal range $0\cdot18–1\cdot18\times10^9$/l). Similar values were obtained on two other occasions. Although the lymphocytes were able to express the activation markers CD25 and HLA-DR after in vitro stimulation with phytohaemagglutinin (table 1), the lymphoproliferative response to phytohaemagglutinin and concanavalin A was impaired (table 2).

These results indicate the presence of impaired cell mediated immune responses in this patient with dyskeratosis congenita. This depression is likely to be contributed by the reversal of the CD4:CD8 ratio and the decrease in absolute numbers of CD4 positive cells. The ability of the patient's T cells to express activation markers upon mitogen stimulation suggests an intact T cell activation pathway.

Discussion

Dyskeratosis congenita is a genetic condition which is ultimately lethal. We have previously reported the characteristic mucocutaneous features in two of our local patients with this disease.[7] Abnormalities in immune function in this disorder is evidenced by a high incidence of unusual infections, and the primary causes of death are infections and carcinoma. In this study, our patient with dyskeratosis congenita and depressed cell mediated immunity developed interstitial pneumonia due to *P carinii* infection; this is an opportunistic infection usually affecting patients with depressed T cell function.

There have been other reports of abnormalities of cell mediated immunity in dyskeratosis congenita. These have included absence or delayed hypersensitivity to a battery of skin test antigens,[3] and impaired response to mitogenic stimulation upon in vitro testing.[8] Our patient also showed impaired lymphoproliferative responses to in vitro stimulation with mitogens (table 2). The mechanism for the impaired cell mediated function has not been delineated. As shown in this study, the presence of a markedly depressed CD4:CD8 ratio (0·38) and decrease in absolute CD4 positive cells are likely contributing factors. The T cell activation pathway appears to be intact since the patient's T cells were able to express normally the activation marker CD25 and HLA-DR (table 1). Expression of these receptors upon lymphocyte activation are an important feature of cellular immunity, as abnormalities in their expression have resulted in severe combined immunodeficiency syndrome.[9 10]

There have also been reports that suggest abnormalities of humoral immunity in patients with dyskeratosis congenita. A wide range of immunoglobulin abnormalities have been reported, including diffuse hypoglobulinaemia,[11] decreased IgG and IgM with normal IgA,[8] normal IgG and IgA with slightly decreased IgM,[12] and increased IgG.[13] Our patient had hypergammaglobulinaemia. With the presence of cell mediated abnormality, as seen in our patient, it is tempting to postulate that the dysgammaglobulinaemia may result from abnormal T cell help, similar to that seen in paediatric acquired immune deficiency syndrome.[14] Interestingly, the genes for dyskeratosis congenita and X linked severe combined immunodeficiency, a condition also associated with depressed cellular and humoral immunity, have both been located on the long arm of the X chromosome. Their locations on the long arm of the X chromosome differ, with the former located in the Xq28[2] band and the latter between region Xq13.1 and Xq21.1.[15]

This study was supported by National University of Singapore Research grants RP 880369 and RP 880337.

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