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In vitro genetically aberrant T-cell clones with continuous growth are associated with atopic dermatitis

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Abstract **Atopic dermatitis is a disease with a genetic predisposition affecting the immune system, with T lymphocytes participating in the immune dysregulation. Most in vitro T lymphocyte studies of atopic dermatitis have focused on antigen-specific T-cell clones. However, antigen-non-specific regulatory T lymphocytes may also take part in the pathway leading to antigen-specific clonal T-lymphocyte proliferation. T lymphocytes from skin biopsy specimens from three patients with severe atopic dermatitis were cultured in the presence of IL-2 and IL-4, but without antigen added. Initially, proliferation was oligo- or polyclonal, but in all cases overgrowth by T cells with clonal chromosomal aberrations was subsequently observed. These abnormal T-cell clones demonstrated continuous growth and complete or partial phenotypic loss of the T-cell antigen receptor complex. In summary, these findings suggest that a subset of aberrant skin-homing T lymphocytes is associated with atopic dermatitis.**

Key words Atopic dermatitis · Chromosome aberration
T cell clones

Introduction

Atopic dermatitis (AD) is considered to be a disease with multifactorial inheritance affecting several parameters of the immune system. Recent reviews have suggested that part of the immune dysregulation is carried by a subset of T lymphocytes [1, 2].

Both antigen-specific and antigen-non-specific T cells take part in the cellular network involved in an antigen-specific immune response. Antigen-non-specific T lymphocytes are believed to mediate help or suppression. We wanted to test the possibility that T-cell growth without antigen added could establish T-cell clones from patients with AD. We thus investigated, in parallel, the growth of T cells from peripheral blood and eczematous skin biopsy specimens from each patient.

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Materials and methods

Patients

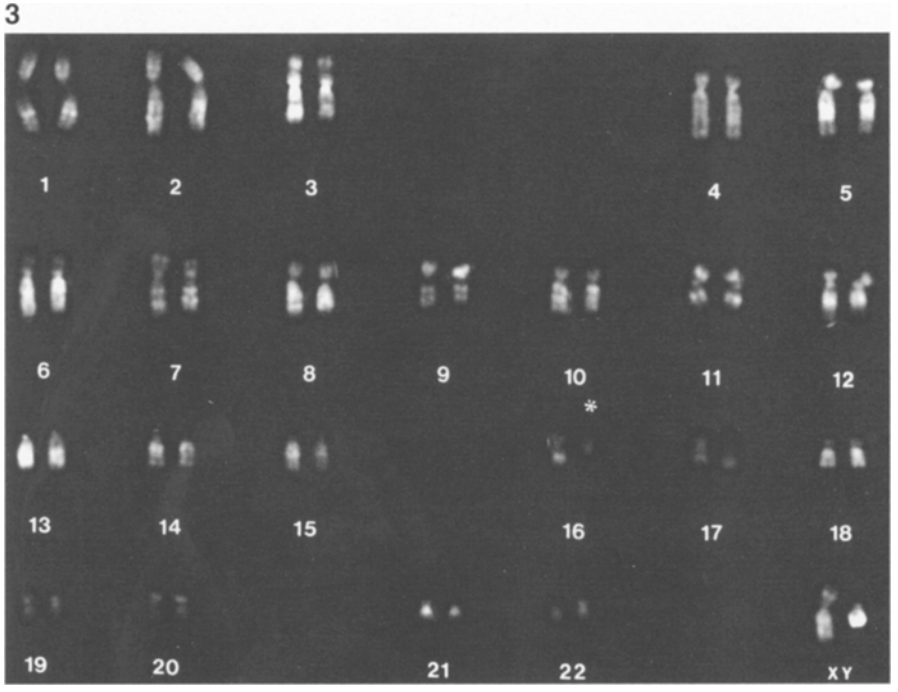
After having given their consent, three male patients with lifelong atopic dermatitis provided skin biopsies and blood samples. Their ages ranged from 20 to 26 years. Their serum IgE levels were 4, 178 and 3544 kU/l. The biopsies were taken from lesions with active non-treated eczema. None of the patients had received systemic therapy for 3 weeks before the biopsies were taken. Blood samples were drawn according to routine techniques.

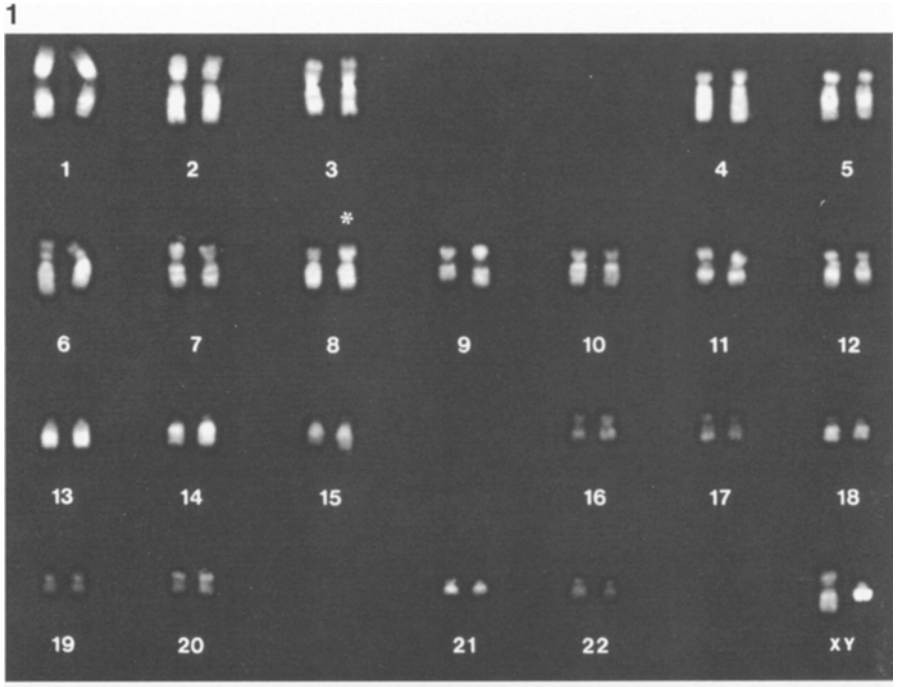
Establishment of T-cell lines

Punch biopsies (4 mm) from eczematous skin were cultured in 5 ml of a medium comprising 90% RPMI-1640 and 10% human AB serum containing 100 U/ml IL-2, 250 U/ml IL-4, 100 U/ml penicillin G and 25 µg/ml streptomycin. When the cell density reached 10^6/ml, the cells were subcultured at a ratio of 1:2. From patient AM a T-cell line, Adam-1, was established. From patient RA, two T-cell lines, Adra-1 and Adra-2, were established from two biopsies taken at different times, and from patient IS a cell line, Adis-1, was established. Mononuclear cells from peripheral blood were isolated by standard Ficoll-Isopaque gradient centrifugation and cultured like the T cells from the eczematous skin at an initial density of 5×10^5/ml.

Phenotyping

The phenotype of the cell lines was established using a panel of monoclonal antibodies and an indirect immune fluorescence technique on living cells. For each antibody 2×10^4 living cells were analysed by flow cytometry. Fluorescence microscopy was also applied to evaluate the stainings. The monoclonal antibodies against epitopes of the T-cell antigen receptor TCR-2 (TCR α/β) had the following specificities: V_β 3.1, V_β 5.1, V_β 5.2, V_β 5.3, V_β 6.7, V_β 8, V_β 12, V_β 13.1, TcR α/β, TCRα and TCRβ (T Cell Di-





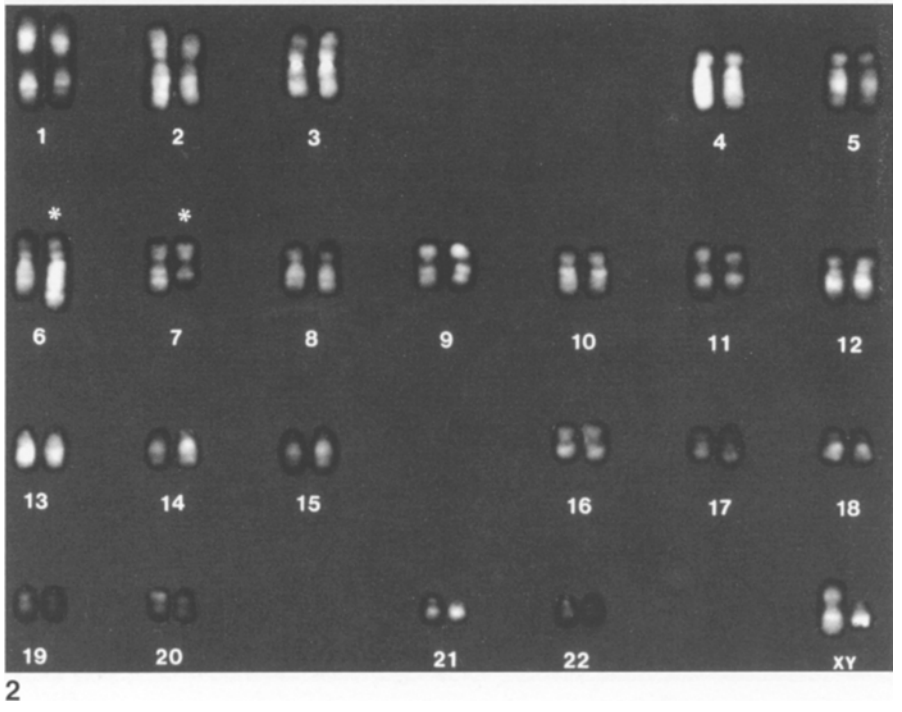


Fig. 1 46,XY,t(8;?)(p12;?) karyotype of Adam-1 cell line

Fig. 2 46,XY,t(6;7)(q27;q22) karyotype of the Adra-1 cell line

Fig. 3 46,XY,del(16)(q11) karyotype of the Adra-1 cell line

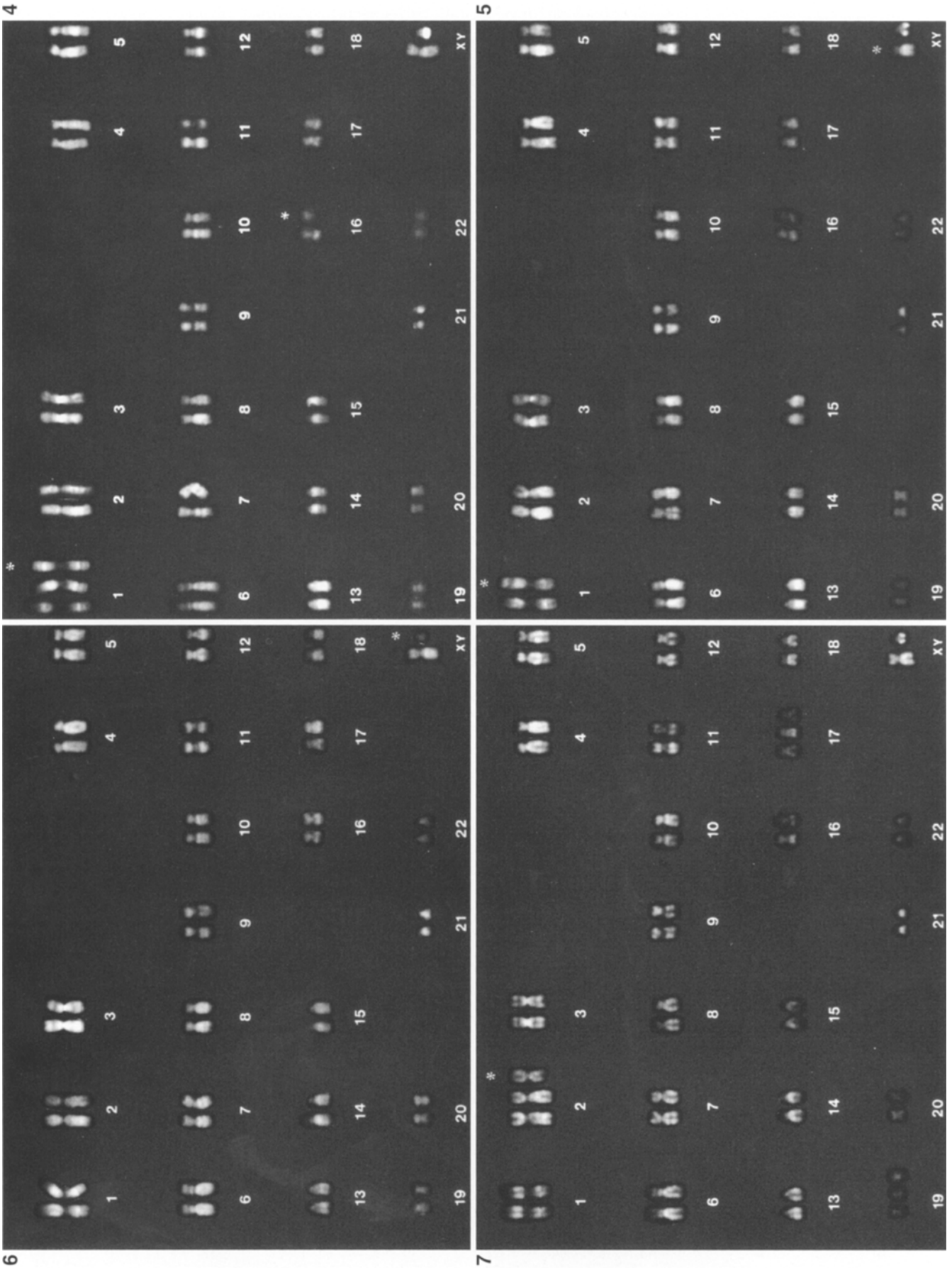


Fig. 4 47,XY,i(1q),del(16)(q11) karyotype of the Adra-1 cell line

Fig. 5 46,XY,t(X;1)(p11;p36) karyotype of the Adra-2 cell line

Fig. 6 46,XY,del(Y)(q12) karyotype of the Adis-1 T cell line

Fig. 7 49,XY,+i(2p),+17,+19 karyotype of the Adis-1 B cell line

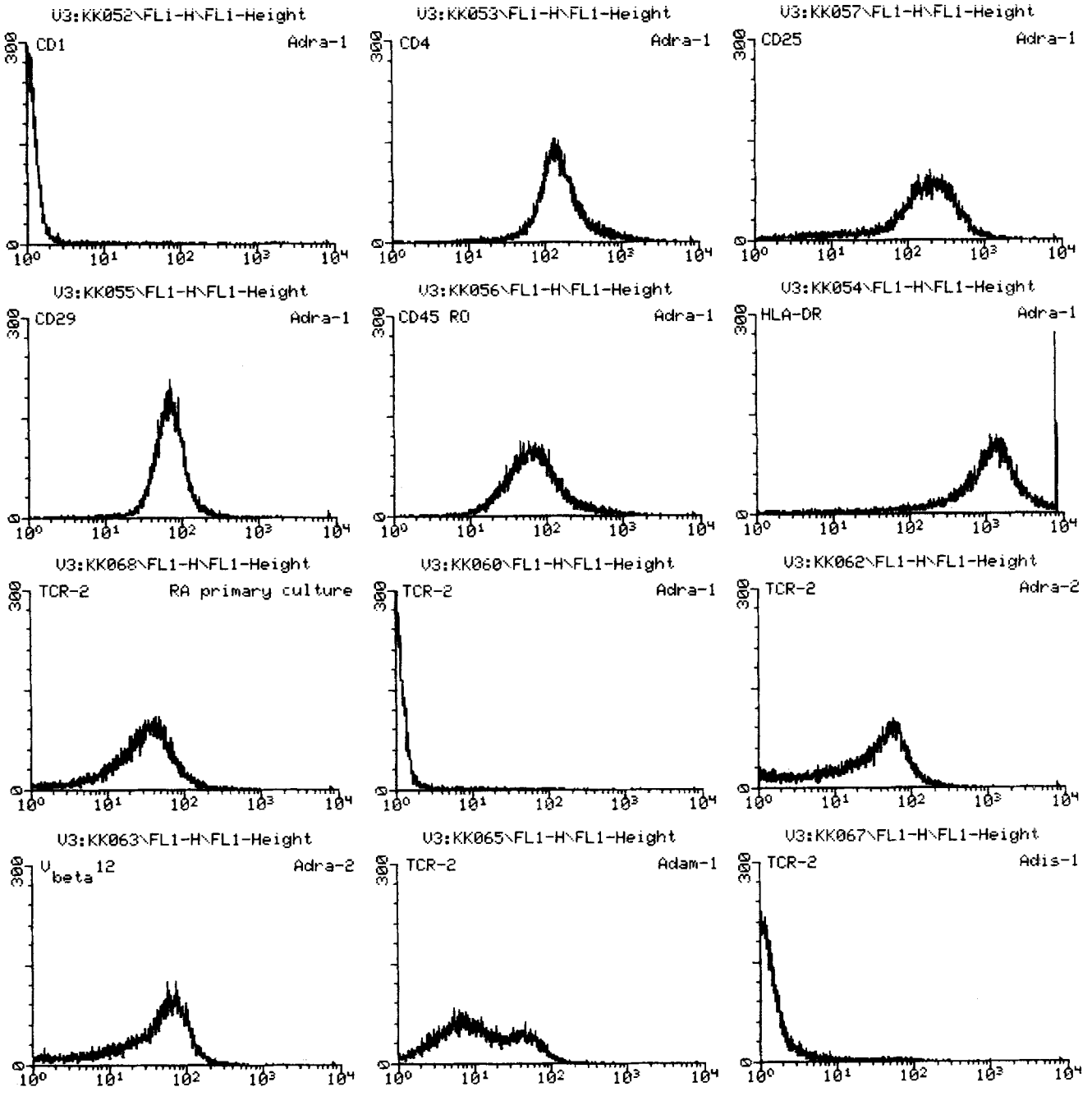


Fig. 8 A flow cytometric analysis of long-term cultured clonal T-cell lines from atopic eczematous skin biopsy specimens

agnostic), V_β 1 and V_β 9 (PharMingen), V_β 2, V_β 3, V_β 13.2, V_β 13.3, V_β 16, V_β 17, V_β 19, V_β 21 and V_β 22 (Immunotech). Monoclonal antibodies to CD1, CD2, CD3, CD4, CD25, CD29, CD45RO and HLA-DR were obtained from Dako.

Seropositive adult T-cell leukaemia virus serum and serum from the three atopic patients were used at concentrations of 1–10% in an indirect immunofluorescence staining protocol on cytocentrifuged and aceton-fixed cell lines.

Karyotyping

Karyotyping with Q banding followed standard procedures. Genetic alterations were monitored when cytogenetically aberrant clones appeared. The karyotypes were established according to the International System for Human Cytogenetic Nomenclature (ISCN) (1985). All cell lines were found to be free of mycoplasma by the Hoechst staining test. T-cell antigen receptor gene rearrangement was analysed as previously described with a panel of restriction enzymes (EcoRI, BamHI, HindIII) and a JCβ1 cDNA probe [3].

Results

Cell culture and cytogenetic analysis

Culture of peripheral blood mononuclear cells resulted in one to four cell population doublings (PD). In contrast to peripheral blood mononuclear cells, T cells from the four

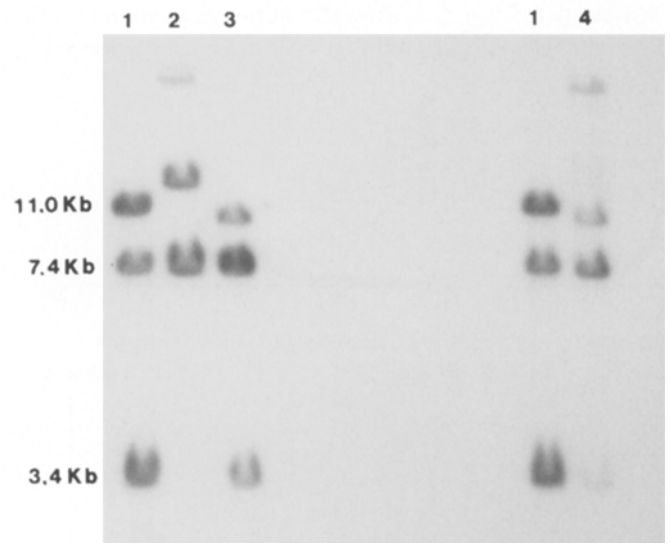


Fig. 9 T-cell receptor gene rearrangements of Adra-1 *(lane 2)*, Adra-2 *(lane 3)* and Adam-1 *(lane 4)*. DNA was digested with HindIII. *Lane 1* shows the germ line configuration of control DNA from peripheral blood

skin biopsies proliferated vigorously in the presence of both IL-2 and IL-4 but without antigen added. Initially, the T-cell cultures were of oligo- or polyclonal origin as evidenced by their reaction with several V_β family antibodies. A positive reaction with a V_β antibody ranged from 0.2% to 10% in the primary culture.

A clonal chromosome aberration was observed in a primary T-cell culture from patient RA. The other skin-derived T-cell lines developed clonal chromosomal aberrations between PD 10 and 70. Metaphases with identical chromosome aberrations are informative concerning clonality. By continuous growth of the T-cell lines only metaphases with clonal chromosome aberrations could be detected. By cytogenetic criteria, the four T-cell lines, Adam-1, Adra-1, Adra-2 and Adis-1, became monoclonal. The PD time for the three former cell lines ranged from 20 to 30 h, whereas the Adis-1 cell line had a PD time of 36 to 40 h.

The karyotype 46,XY,t(8;?) of the Adam-1 cell strain is shown in Fig. 1. This cell line has made more than 250 PD and can thus be considered continuous (immortal). Between PD 30 and 70 the Adra-1 cell line consisted of two cytogenetically different T-cell clones with the karyotype 46,XY,t(6;7) shown in Fig. 2, and 46,XY,del(16q) shown in Fig. 3. The 46,XY,del(16q) T-cell clone then overgrew the 46,XY,t(6;7) clone and had to date exceeded 300 PD. The two cytogenetically different Adra-1 T-cell clones also differed in their T-cell antigen receptor expression because only the Adra-1 46,XY,del(16q) cell line expressed V_β 12 until approximately 250 PD. The latter cell strain also demonstrated additional genetic instability giving rise to a subclone with a 47,XY,+i(1q),del(16q) karyotype (Fig. 4).

In a primary T-cell culture from patient RA the 46,XY,t(X;1) karyotype (Fig. 5) was seen in 6 of 276 metaphases. This cytogenetically aberrant T-cell clone, Adra-2, had to date made 150 PD. The karyotype of the Adis-1 T-cell line is shown in Fig. 6. Besides this T-cell line, a B-cell line with the karyotype shown in Fig. 7 appeared during culture.

The requirement for IL-2 and IL-4 for continuous growth was investigated by withdrawing one of the two cytokines from the Adam-1 and the Adra-1 cell lines at different times. To date these two cell lines have been shown to require both IL-2 and IL-4 for continuous growth.

Phenotyping of the skin-derived T-cell lines Adam-1, Adra-1, Adra-2 and Adis-1 basically showed the phenotype of activated mature T cells: $CD2^+$, $CD3^+$, $CD4^+$, $CD25^+$, $CD29^+$, $CD45R0^+$, HLA-DR^+, TCR-2^+, CD1 antigens were not detected. However, upon continuous growth, a loss of phenotypic markers occurred as shown by flow cytometry (Fig. 8). The Adra-1 and Adis-1 T cell lines lost surface expression of CD3 and TCR-2. Partial loss of TCR-2 was observed in the Adam-1 cell line. In the Adra-2 cell line, only 10% of the clonal T cells at PD ~ 150 had lost TCR-2 expression compared with the primary culture.

No human T-cell lymphotropic virus-1 (HTLV-1)-related antigens were detected and no specific reaction of patient sera was observed towards the skin-derived T-cell lines.

T-cell receptor gene rearrangement

Clonality based upon cytogenetics demonstrating clonal chromosomal aberrations was confirmed by T-cell receptor gene rearrangement analysis. All cytogenetically different T-cell clones also demonstrated different T-cell receptor gene rearrangements. Although the Adra-1 and Adra-2 T-cell lines during a period in culture both expressed V_β12, T-cell receptor gene rearrangement showed that they are different T-cell clones (Fig. 9).

Discussion

The results reported here demonstrate that continuous T-cell lines can be established from atopic eczematous skin biopsy specimens. During continuous culture these T-cell lines developed clonal chromosomal aberrations.

It is believed that normal T lymphocytes like other somatic human, diploid cells demonstrate replicative senescence in tissue culture [4]. This limit, known as the Hayflick limit, occurs after approximately 30 to 50 PD [4, 5]. The skin-derived T-cell lines described here exceeded by far the Hayflick limit, being effectively immortal. As a consequence – or cause – these continuous T-cell lines developed clonal chromosomal aberrations somehow reflecting a defective cell cycle control. Immortality in tissue culture and the development of clonal chromosomal aberrations are generally believed to reflect either the presence of an immortalizing exogenous virus or progression towards malignancy. Because no HTLV-1-related antigens were detected in the continuous T-cell lines and

because none of the patient sera specifically reacted with the cell lines, immortalization with an exogenous agent seems less likely. This is compatible with the observation that peripheral blood T cells are not immortalized by addition of γ-irradiated skin-derived T-cell lines. Immortalization and development of clonal chromosomal aberrations are more likely to be an intrinsic property of a subset of cultured T lymphocytes from atopic skin, i.e. there is a predisposition for genetic instability and continuous growth in tissue culture. The lymphocytic infiltrate in AD is reactive, and only very rarely does AD develop into malignant lymphoma [2]. Continuous T-cell lines from patients with AD have in vitro parameters resembling 'malignant' rather than reactive T cells. However, preliminary experiments have indicated that peripheral blood cells from AD patients in vitro can eliminate these genetically aberrant T cells.

One previous study has demonstrated T-cell growth without antigen added from eczematous skin biopsy specimens [6]. No cytogenetic study was performed during this short-term culture period (approximately 7 PD). We have previously established continuous T-cell lines from patients with cutaneous T-cell lymphoma [7–9]. Some of these T-cell lines also developed clonal chromosomal aberrations, and T cells with the ability to develop clonal chromosomal aberrations have been named genotraumatic T cells [8, 9]. This implies that genetic instability is a central feature for these T cells. Both malignant and non-malignant continuous T-cell lines can be established from patients with cutaneous T-cell lymphoma [8, 9]. The continuous in-vitro-cultured atopic T-cell lines described here are non-malignant genotraumatic T cells.

The continuous autonomous paracrine growth of atopic T-cell lines followed by the development of clonal chromosomal aberrations might indicate that these T cells were selected in a faulty manner during thymic maturation and differentiation. The results of this investigation suggest that a subset of aberrant skin-homing T cells is associated with AD.

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