Lack of Membrane Expression of Interleukin-2 Receptor α Chain (CD25) in Mycosis Fungoides: Application of Laser Scanning Cytometry for Phenotyping of Skin Infiltrating Lymphocytes

To the Editor:

Interleukin-2 (IL-2) is centrally involved in T cell proliferation and is alone able to support the cell cycle progression of T cells (Cantrell and Smith, 1984). Besides its pivotal role in normal immunologic response, IL-2 has been shown to be an important cytokine perpetuating the growth of leukemia and lymphoma cells, such as adult T cell leukemia/lymphoma. Recently, a chimeric fusion protein toxin DAB486IL-2, which is able to selectively kill the cells expressing the IL-2 receptor, has been proposed as a therapeutic modality for certain leukemias and lymphomas, including mycosis fungoides and Sezary's syndrome (Foss *et al*, 1994).

Measuring the membrane expression of IL-2 receptor is important for predicting the responsiveness of the cell for IL-2 and the effect of IL-2 fusion toxin therapy. This is usually achieved by flow cytometry following the labeling of viable cells with CD25 (interleukin-2 receptor α chain, Tac, p55) antibody; however, in the case of cutaneous lymphomas flow cytometry has been difficult to apply due to the problems in obtaining a sufficient number of lesional lymphocytes for analysis. Immunophenotyping on skin sections cannot fully replace flow cytometry because it is difficult to differentiate between the cytoplasmic and membrane expression of the antigen.

In this communication we show that laser scanning cytometry (LSC) (Kamentsky and Kamentsky, 1991; Clatch *et al*, 1998) may be applied to measure membrane antigen expression in lesional lymphocytes of mycosis fungoides and demonstrate the application of this method for CD25 antigen determination. LSC is a microscope-based technique comparable with flow cytometry where fluorescence is quantitatively estimated in the cells attached to the microscope slide (Kamentsky and Kamentsky, 1991). With this technique, that has already been used for clinical immunophenotyping in hematology, it is possible to analyze small samples (down to several hundred cells), to rescan the same sample several times following different stainings, and to visualize the cells of a given phenotype (Clatch and Foreman, 1998).

Two 4 mm biopsies of lesional skin were obtained from 12 patients (age 68–83, five men, seven women) with histopathologically confirmed mycosis fungoides in plaque/tumor stadium. The biopsies were cut into small fragments and incubated in RPMI1640 with 10% fetal calf serum for 48 h in a humidified incubator at 37°C in an atmosphere of 5% CO_2. The cell aggregates and tissue fragments were filtered and the cells that migrated from the biopsies (mainly leukocytes) were labeled with the FITC-labeled CD25 antibody (Dako, Glostrup, Denmark), PE-labeled CD4 antibody (Dako), and the CD45 antibody conjugated with PeCy5. The cells were placed into chamber slide and incubated for 30 min at 4°C to prevent capping. After washing off the unbound antibody the cells were scanned in LSC with a 488 nm argon laser as an excitation source. A typical result of the phenotyping from one patient is shown in **Fig 1**(***A***, ***B***). Among infiltrating leukocytes ($CD45^+$ cells) the majority (70%–95%) were $CD4^+$ lymphocytes that did not express CD25. To determine the cytoplasmic expression of CD25 the cells attached to the slides were fixed in cold acetone for 15 min and re-labeled with CD25-FITC and CD4-PE. In three cases a population of $CD25^+$ could be discerned suggesting a cytoplasmic but not membrane expression of CD25 (**Fig 1*C***).

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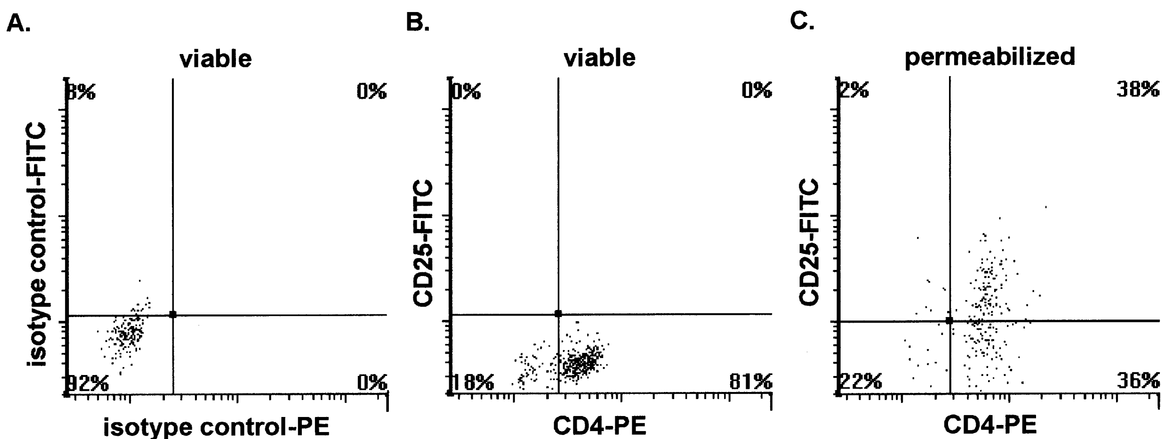

Figure 1. Immunophenotyping of lesional lymphocytes from a patient with mycosis fungoides. The viable lesional lymphocytes (*A*, *B*) were labeled as indicated in the text and scanned. The isotype control staining in (*A*) defines the background fluorescence. In (*C*), the cells were permeabilized and restained with the antibodies to detect the cytoplasmic antigen. In this case, the cytoplasmic, but not the membrane expression of CD25 is seen.

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Histochemical studies revealed that CD25 expression in lesional skin in mycosis fungoides occurs in up to 50% of all cases (Oishi *et al*, 1994; Bagot *et al*, 1996; Stefanato *et al*, 1998). The conclusion from our study, although limited by a small number of patients, is that membrane CD25 (and hence IL-2R) expression is a very rare phenomenon in mycosis fungoides and cannot be assumed to occur in the presence of the cytoplasmic expression of this molecule. Although not investigated here, it is possible that IL-2R expression is lost during progression of mycosis fungoides and other cutaneous lymphomas (Boehncke *et al*, 1993; Wasik *et al*, 1996). It is thus unlikely that IL-2 plays an important role in the growth of transformed lymphocytes in mycosis fungoides, a conclusion that supports previous results (Boehncke *et al*, 1993). Because membrane expression of CD25 is a prerequisite for successful therapy with DAB486IL-2, these results indicate that phenotyping of viable lesional lymphocytes should be undertaken in all patients where such a therapy is planned. Finally, LSC seems to be a promising method enabling membrane phenotyping of lesional lymphocytes in patients with cutaneous lymphoma.

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Marie Unna Hereditary Hypotrichosis Gene Maps to Human Chromosome 8p21 Near *Hairless*

To the Editor:

Individuals with Marie Unna hereditary hypotrichosis (MUHH) (MIM: 146550), a rare autosomal dominant disorder, have anomalies affecting both hair density and hair shafts (Unna, 1925). About a dozen families of Caucasian origin with the trait have been described in the literature. Affected individuals are born with sparse, normal-to-coarse scalp hair, which becomes more sparse, coarse, and wiry during childhood. Hair shafts are deeply pigmented, large in diameter, and kinked at random, odd angles, which causes them to stand out from the head. Although severity of scalp hypotrichosis is variable, all affected individuals have sparse-to-absent body hair, eyelashes, eyebrows, and secondary sexual hair. In most affected individuals, scalp hair is progressively lost starting at puberty or beyond, with the most severely affected individuals manifesting near total loss of crown and ophiasis hair, retaining a narrow rim of sparse, coarse hair in a fringe around the scalp periphery in a tonsorial pattern. There is no histologic evidence of scarring, and scalp hair can be extracted by a light hair pull.

Here we report a large Caucasian kindred of MUHH with multiple affected individuals, and the mapping of the gene by genetic linkage analysis. The pedigree and the proband are shown in **Fig 1(*a*)** and **Fig 1(*b*)**, respectively. Phenotype of all individuals was confirmed by one of us (JRR). Affected individuals in the pedigree have typical features of the MUHH trait. Similar to previous reports (Solomon *et al*, 1971), we found wide spacing of the upper incisor teeth in 50% of the members of our family. There is no facial dysmorphism. Intellectual and sexual development is normal.

We initiated genetic linkage studies of the pedigree, shown in **Fig 1(*a*)**, by using polymorphic microsatellite markers of Genethon map (Dib *et al*, 1996) from the genomic regions with relevance to abnormalities in hair follicle density and structure. Computer simulation studies using SLINK program[1] showed that the kindred collected for this study had the potential to generate a LOD score (Lathrop and Lalouel, 1984) of more than 3.0 (average maximum 2.44 with a standard deviation of 0.99 and a theoretical maximum of 4.43). After genotyping six genomic candidate loci with multiple

Table I. Two-point LOD score values between the disease locus (MUHH) and Chromosome 8p microsatellite markers

	LOD score at θ^a =					
Marker	0	0.05	0.1	0.2	0.3	0.4
D8S258	0.28	0.34	0.37	0.35	0.27	0.15
D8S298	3.81	3.52	3.21	2.52	1.73	0.84
D8S1786	3.61	3.3	2.97	2.26	1.48	0.66
D8S1733	2.53	2.29	2.03	1.46	0.84	0.25
D8S1752	3.31	3.02	2.72	2.06	1.34	0.59
D8S1734	0.95	0.86	0.76	0.57	0.37	0.18
D8S1739	$-\infty$	1.3	1.44	1.26	0.83	0.34
D8S1771	1.11	1.02	0.92	0.69	0.42	0.15

[a]Recombination fraction from MLINK program.

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