tients with the macrophage pattern developed GvHD compared to 4/19 who did not have macrophage predominance. Of 14 patients who developed GvHD, 10 had the macrophage pattern compared to 1/16 who had no GvHD. Of the 14 patients who are now alive without GvHD, only 1 had the macrophage pattern, whereas 4/5 patients who died of GvHD had shown strong macrophage predominance.

Discussion. CML-patients with a predominance of free and clustered macrophages in peripheral blood cultures have a high incidence of GvHD. Although these excess macrophages are not the cause of GvHD, since the association with GvHD was not 100%, it is conceivable that they favor the development of GvHD if there is a minor histoincompatibility between patient and donor, which is not detected with the available techniques for HLA matching. Macropages are antigen presenting cells. Since they are more radioresistant than other immunocompetent cells, their excess may favor recognition of recipient antigens by donor T-lymphocytes even after BMT, and thus enhance GvHD. The predictive value of macrophage predominance in culture may be of value in the choice of patients who should be given a T-depleted rather than an unseparated bone marrow graft. It can be speculated that treatment of the recipient aiming at reduction of functional macrophages, e.g. with indomethacin, might reduce the risk of GvHD. It is striking that none of the 6 patients who had no growth at all in peripheral blood cultures developed GvHD; 5 of them had an uneventful clinical course and are alive and well. This observation suggests that BMT is associated with the fewest complications if the patient has a hypoplastic marrow at the time of transplantation. Eradication of the Philadelphia positive clone to the maximum possible extent – and thus eradication of excess macrophage activity – appears to be desirable prior to BMT for CML.

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Thymine dimer repair in fibroblasts of patients with dysplastic naevus syndrome (DNS)

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Summary. Dysplastic naevus syndrome (DNS) is frequently observed in association with familial melanoma and xeroderma pigmentosum (XP), but the role of UV-light in the development of DNS has not been elucidated. Previous work has shown that UV-induced unscheduled DNA synthesis is associated with the early loss of antigenicity observed in immunoassays using a monoclonal antibody specific for thymine-thymine dimers. We now show that the rate of loss of antigenicity, which reflects the relative amount of bound antibody, observed during the first 60 min following 10 Jm^{-2} UVC irradiation is significantly reduced ($p = 0.02$) in cultures of fibroblasts from 7 out of 8 DNS patients compared with the results from cells of a group of 30 healthy volunteers. This observation suggests an early event in excision repair is altered in the majority of DNS patients.

Key words. Dysplastic naevus syndrome; DNA-repair; cancer genes; familial malignant melanoma; monoclonal antibodies specific for UV-dimers.

Dysplastic naevus syndrome (DNS) is a preneoplastic melanocyte abnormality which occurs in both familial and sporadic forms.

Formal genetic analysis has demonstrated that members of certain families inherit a dominant gene with a high penetrance that leads to the development of the syndrome, which is characterized by multiple moles having an unusual variety of colors, sizes and shapes[1-3, 7-10, 15]. The pathological features arise in adolescence and continue to appear even after the age of 35. Dysplastic naevi patients were found to carry a high risk of developing a melanoma, the fatal type of skin cancer. The mechanisms leading to atypical moles and melanoma are at present unknown[1, 3, 7, 15]; however, sunlight seems to aggravate the course of the disease. Fibroblasts from patients with DNS are reported to be unusually sensitive to ultraviolet (UV) light[8, 10, 15, 17, 21, 23].

We have recently described in detail[19] an EIA that employs a monoclonal antibody specific for UV-induced thymine dimers in single-stranded DNA[27]. The assay was used to monitor changes in the antigenicity occurring in the DNA of UV-irradiated cells as a function of time after UV-irradiation. Our results confirmed the observation of Clarkson et al.[4] that large loss of antigenicity occurs during the first 30 min after irradiation in excision proficient cells. Cell strains that show impaired unscheduled DNA synthesis after UV also show a reduction in the rate of loss of antigenicity. In the present study we have used the assay to examine the early steps of excision repair in fibroblasts of patients with DNS and healthy controls.

Materials and methods. Cell cultures. Thirty control samples were obtained from normal, healthy male and female volunteers who were 25–27 years of age. These included 20 blood samples and 10 skin biopsies[19]. Biopsies from 8 patients with dysplastic naevus syndrome were obtained from the departments of Dermatology of the Kantonsspital, Basel, and the University Hospital of Zürich. All biopsies were taken from sun-shielded and non-malignant parts of the skin.

Growth and irradiation of cells. Biopsy samples from sun-shielded parts of the trunk unaffected by disease were minced and teased apart under sterile conditions, and explant cells were cultivated in MEM supplemented with 10% newborn calf serum, 1% non-essential amino acids, 2 mM L-glutamine and 2% vitamins. No antibiotics or antimycotics were added, and the medium was changed every other day. After 4 weeks, the cultures were divided and placed in 75-cm^2 flasks (Falcon Plastics) for 2 additional weeks until the cells were confluent. The cultures were trypsinized and the cells

were divided into 5 aliquots (petri dishes; 60 × 15 mm, Falcon) with fresh medium and were grown at 37 °C with 5% CO_2 in a humidified incubator.

Cells were irradiated with a Phillips 6V germicidal UV-lamp emitting predominantly 254-nm radiation at 0.2 Jm^{-2} s^{-1} at a distance of 38 cm. Each culture was divided into 5 equal aliquots (1 non-irradiated control and 4 irradiated). After pouring off the medium from the petri dishes, the samples were irradiated with 10 Jm^{-2}. Fresh medium was added immediately and the cells were incubated at 37 °C for various times before samples were assayed. At each time point the repair processes were stopped by removing the medium and freezing the aliquot of cells with liquid nitrogen.

Extraction and preparation of DNA. DNA was extracted by a phenol/chloroform method which has been described elsewhere [11].

DNA quantitation. DNA was quantitated photospectrometically at 260 nm [5]. Five µl of each DNA sample were dissolved in 995 µl H_2O and transferred into a quartz cuvette (pathway for the light = 10 mm). The spectrophotometer was adjusted to zero with water and the optical density ($OD_{260\,nm}$) was read at 260 nm. The concentration of DNA in micrograms per microliter is 10 times the $OD_{260\,nm}$ reading.

Purity of DNA was assessed by a second reading at a wavelength of 280 nm and the ratio of the two measurements was calculated. The ratio should range between 2.0 and 1.6 to ensure that the DNA is protein-free.

Enzyme immunoassay. EIA was performed in 96 multiwell plates (Nunc). To obtain ss-DNA, 0.5 ml of each sample was boiled for 10 min and 2% agar-agar (Fluka) was added to optimize binding to the multiwell plate [16]. Aliquots of 10 µl per well were added and incubated overnight at 37° C. Non-absorbed DNA was removed by washing 5 times with washing buffer (PBSAT = PBS'A' containing 0.02% Tween) and non-specific binding sites were blocked by the addition of the blocking buffer (0.0425 M Na_2CO_3 + 0.02 M $NaHCO_3$ + 2% skim milk powder, pH = 10.6) and incubating for 90 min at room temperature in the dark. After washing 5 times with PBSAT, wells were incubated for an additional 90 min at room temperature in the dark with anti-UV ss DNA-1 monoclonal antibody [22] diluted in coating buffer (blocking buffer without skim milk), washed 5 times with PBSAT and incubated similarly with a peroxidase conjugated anti-mouse antibody. Wells were washed 5 times with deionized water and incubated for 30 min at room temperature in the dark with 50 µl peroxidase reagent (40 mg 1,2-phenylenediamine in 100 ml 0.2 M citric acid, 0.1 M NaH_2PO_4, pH = 5.0 to which was added 40 µl H_2O_2). The reaction was stopped after 30 min with 25 µl of 2.5 N H_2SO_4 and the optical density of the contents of each well was measured at 490 nm. Individual samples were assayed in triplicate unless otherwise specified.

Results. Figure 1 shows the mean loss of antigenicity ± standard error [20] for samples from 30 healthy volunteers exposed to 10 Jm^{-2} UVC irradiation. The calculation for the loss of antigenicity has been described elsewhere [19]. It is the percentage of decrease of antibody binding sites (conformational DNA changes caused by thymine dimers) on the DNA measured as a percentage of antigenicity.

The standard deviation for three independently repeated cultures from the same individual was +/− 6.5%. For the triplicated readings of bound antibody the standard deviation was +/− 10%; this gives the standard error of the method [19]. The standard deviation for the DNS patients ranged from +/− 5.4 to +/− 7.1, but was not significantly different from those of the healthy controls.

A rapid loss of antigenicity within the first 30 min of incubation is followed by a slower rate of loss. In contrast, cells from an XP patient, sister of DNS patient BS 3, showed a slow rate of loss of antigenicity associated with the excision repair deficiency of XP.

The kinetic profiles of the cultured fibroblasts from 8 DNS patients (table) were compared with these controls (fig. 2). With the exception of patient ZH 9, all showed a rate of loss of antigenicity that was significantly less at times 10, 30 and 60 min than that of the healthy control group ($p = 0.05$; Student's t-test). At times later than 60 min most DNS samples did not differ from the healthy controls.

Comparison of the results from individual DNS patients showed considerable variability in the response at early times. Interestingly, patient BS 3 showed a similar kinetic response during the first 30 min of incubation to that of his XP sister. The kinetic response of patient ZH 9 was different from all other DNS patients ($p = 0.005$).

Discussion. The familial displastic naevus syndrome is a dominant trait with extensive heterogeneity and variable expressivity. It appears under various designations in the literature: B-K mole syndrome, familial atypical multiple melanoma (FAMM) syndrome or large atypical mole syn-

Clinical observations in the investigated DNS patients.

Patient code	Dysplastic naevus synd.	Melanoma	Other tumors	Tumor cases in the family
ZH 9	+	−	−	Father with melanoma
ZH 36	+	+	−	no
ZH 54	+	−	−	no
ZH 71	+	−	−	no
BS 1	+	+	−	no
BS 2	+	+	Multiple basalioms	Fam. DNS[a]
BS 3	+	−	−	Sister w. XP[b]
BS 4	+	+	−	no

[a] Familial dysplastic naevus syndrome; [b] Xeroderma pigmentosum.

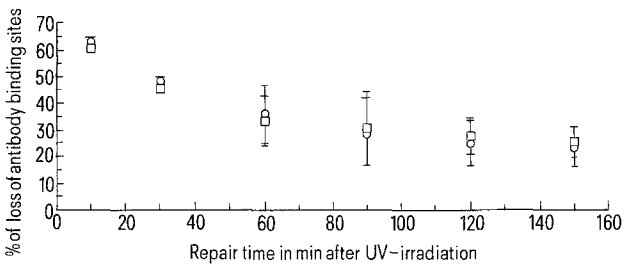

Figure 1. Mean values of the loss of antibody binding sites (loss of antigenicity) in lymphocytes from 20 healthy blood donors and skin biopsies from 10 volunteers. The means of the 20 lymphocyte samples are indicated as □; the means of the 10 skin biopsies are marked as ○. The mean values are shown ± SE.

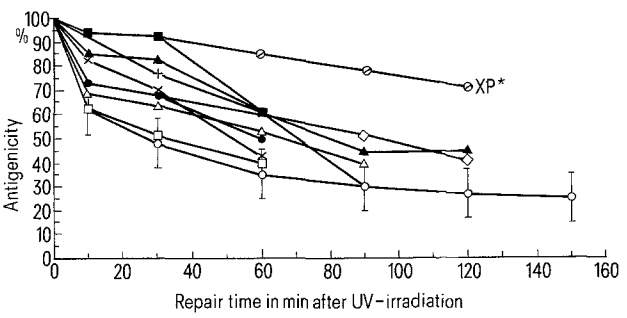

Figure 2. Loss of antibody binding sites (loss of antigenicity) during the first 150 min after an UV-dose of 10 Jm^{-2}. Single patients are marked: □ zh9, △ zh36, ◇ zh54, + zh71, × BS 1, ● BS 2, ■ BS 3 and ▲ BS 4. The mean of 30 healthy controls (○) is shown ± SE. * XP: Xeroderma pigmentosum affected sister of patient BS 3.

drome [3, 7, 10, 15]. Similar dysplastic naevi also occur in association with sporadic melanoma or without melanoma. Since moles are predominantly found on the skin of sun-exposed regions of the patients, a systemic hypersensitivity to ultraviolet irradiation and ultraviolet-mimetic carcinogenes has been postulated [15]. However, because of the small number of patients examined and some contradictory results, final conclusions cannot yet be drawn.

We have found that the cells from a majority (7 out of 8) DNS patients show an abnormal response to UV expressed as a slower loss of antigenicity with a monoclonal antibody to thymine dimers. This difference is observed within the first hour after UV-irradiation. By 90 min the repair kinetics of DNS cells and normal cells are identical. These results are consistent with three earlier studies of DNA repair [15, 17, 21]. Smith et al. [17] found no differences when the repair kinetics of DNS and normal cells was examined 2 h after 15 Jm^{-2} irradiation. Similarly, Ramsey et al. [17] found no difference in unscheduled DNA synthesis in DNS and control cells at 4 h after 20 J m^{-2} irradiation although DNS cells showed hypersensitivity to UV in terms of colony forming ability. Perera et al. [15] found DNS cells to be hypermutable by UV. The study also showed that DNA synthesis was inhibited more or less in DNS cells than in control cells during the first hour following 5 Jm^{-2} irradiation, but at later times, no differences were observed. Together these results suggest that cells of DNS patients have an abnormality of DNA metabolism that results in a transiently slower response to ultraviolet damage.

The increased UV-sensitivity [19, 21] and hypermutability of DNS cells following UV-irradiation are observed also in cells of XP patients [14] characterized by defective excision repair of DNA damaged by UV-irradiation [2]. Patients with both of these diseases also share a marked increase in the incidence of cutaneous melanoma, intraocular melanoma and breast cancer as well as of cancer of the respiratory, gastrointestinal and lymphatic systems. The relationship between DNS and XP is emphasized in the family of DNS patient BS 3 whose sister had XP. In the immunokinetic assay cells from both siblings show the same abnormal response during the first 30 min, after which the DNS samples return to the normal type response.

Dysplastic naevi are both markers and precursors of malignant melanoma [1, 4, 7, 11, 21]. Cells grown from biopsies of unaffected regions of the skin of 10 melanoma patients showed a significantly reduced response in the immunokinetic assay when compared with cells from normal volunteers, not in the 0–30-min intervals as with DNS, but at times greater than 30 min [19]. In the same study the response of cell cultures derived from patients with basal cell carcinoma (bcc) was indistinguishable from that of normal cells. The immunokinetic assay appears to be able to distinguish between DNS, melanoma, bcc and XP in the majority of cases, although the heterogeneity of the response within each group means that the method is useful as an indicator of disease and not as a diagnostic test. The association of a rapid loss of antigenicity with a capacity for unscheduled DNA synthesis seen in this comparison of normal and XP cell lines [19] strongly suggests that a reduction in immunogenicity reflects a change in topology of the thymine dimer as an early event in excision repair. Paterson and co-workers [12] have suggested that excision of the intradimer phosphodiester bond is an earlier event in excision repair that induces a conformational change, preparing the dimer as a substrate for a generalized 'bulky lesion repair complex'. However, since XP group C showed reduced loss of antigenicity but normal dimer phosphodiesterase activity [13] it is probable that those events are unrelated. At the present time we tentatively conclude that the altered immunokinetics frequently seen in DNS cells and melanoma imply some abnormality in an early step of DNA repair analogous to that seen in XP but not observed in cells from bcc patients.

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