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Letters

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Increased Risk of Malignancy for Patients with Chronic Granulomatous Disease and its Possible Link to the Pathogenesis of Cancer

E.A.M. Weel,[1] W.K. Redekop[2] and R.S. Weening[1,3]

[1]Emma Kinderziekenhuis and [2]Department of Clinical Epidemiology and Biostatistics, Academic Medical Center, Amsterdam; [3]Laboratory for Experimental and Clinical Immunology, University of Amsterdam, The Netherlands

CHRONIC GRANULOMATOUS disease (CGD) is a rare inherited disorder of non-specific immunity, characterised by recurrent bacterial and fungal infections. Phagocytic leucocytes of patients with CGD fail to generate superoxide (O_2^-), which is required for efficient killing of phagocytosed microbes. This is due to a defect in a NADPH oxidase found in phagocytes [1], which consists of at least four components, one of which is encoded by an X-linked gene.

Although several immune system disorders are associated with an increased incidence of malignancy, no association between CGD and malignancy has yet been described. We have diagnosed 3 CGD patients with a malignancy. Patient A is male, born in 1963, and diagnosed in 1963 with a retinoblastoma ocula dextra. In 1977 an autosomal recessive form of CGD (p47-phox deficiency) was identified. Patient B is female, born in 1965. In 1978 an autosomal recessive form of CGD (p47-phox deficiency) was diagnosed. In 1991, a malignant melanoma of her right shoulder was radically excised (Clark level IV, Breslow 1 mm). Patient C is male, born in 1968. In 1975 a rhabdomyosarcoma of the liver was diagnosed. He was treated with cytostatics. In 1978 X-linked CGD (gp91-phox deficiency) was diagnosed [2].

The family histories of these 3 patients did not reveal other patients with CGD or cancer at young ages in first-degree relatives.

Correspondence to R.S. Weening.
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In total, 42 patients with CGD have been identified in The Netherlands (prevalence 1:300000), while the reported prevalence in the U.S.A. is 1:500000 [3]. We compared the risks of cancer in this group with the risks in the normal Dutch population. The Netherlands 1990 incidence rates for all forms of cancer were used in this study. An estimate of the overall relative risk (RR) was calculated, using stratification by age (5-year intervals) and sex. As a method of assessing RR, we performed analyses of the incidence density ratios. Exact confidence intervals were calculated, because the number of observed events in the CGD group was small.

Data from The Netherlands Cancer Registry [4] showed that 8321 cases of all cancers occurred in 1990 (ages 0–49; incidence: 76 per 100000 person-years). The number of cases expected to occur in the CGD group on the basis of 1990 incidences is 0.2173. The RR is 13.80 (95% confidence intervals: 2.84, 40.33).

There are three points to be made regarding this analysis. First, the cancers in the CGD group occurred at relatively young ages. Because there is no reason to restrict the analysis to younger age groups, all person-years contributed by the CGD group were included. If only the patient-years contributed by the CGD patients in the age groups up to 25—29 years had been analysed, the overall RR would have been much higher. Second, use of the 1990 incidence rates probably resulted in an overestimate of the background risks experienced by these patients over their periods at risk. Third, the incidence of all cancers was included because there is no reason to link specific forms of cancer (melanoma, retinoblastoma and rhabdomyosarcoma) with CGD. If the population incidences for these three types had been used, the estimate for the RR would have been 93.36 (95% confidence intervals: 19.23, 272.80).

There are several reports in the literature indicating that oxygen radical formation plays a role in both the induction of DNA damage [5, 6] and tumour cell killing [7]. Weitberg and associates showed that oxygen radicals from human phagocytes induced cytogenetic damage in cultured mammalian cells [8]. Furthermore, a strong correlation exists between mutagenesis and carcinogenesis [9]. Another indication for the involvement of phagocytes in cancer comes from Clark and Klebanoff, who showed that the neutrophils of CGD patients are unable to lyse tumour cells by antibody-dependent cell cytotoxicity (ADCC) [7]. Superoxide is an essential intermediate for ADCC, because inhibitors of the NADPH oxidase inhibit ADCC tumour cell killing by neutrophils [10]. These studies indicate that oxygen radicals are able to induce cancer and might be involved in the defence against tumours. CGD is a heterogenous group of disorders in which mutations occur in one of the several genes, all leading to a partial or complete deficiency of the NADPH oxidase and resulting in defective oxygen radical formation during activation of phagocytes (neutrophils, monocytes, macrophages, eosinophils). The observed malignancies in these patients are also heterogeneous. Therefore, no correlation between these malignancies with the genetic background of CGD could be established. However, our findings are suggestive that these tumours, at relatively young ages, are related to this congenital phagocytic defect and may be caused by the defective tumour cell killing.

We plan to test this hypothesis in NADPH oxidase knock-out mice.

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Home Therapy with Autologous Tumour-infiltrating Lymphocytes and Subcutaneous Interleukin-2 in Metastatic Melanoma

M.-H. Tessier,[1] M.-C. Pandolfino,[2] F. Jotereau,[2] D. Boudart,[3] P. Litoux[1] and B. Dréno[1]

[1]Department of Dermatology, C.H.U. Hôtel-Dieu; [2]I.N.S.E.R.M. U 211; and [3]C.R.T.S. (Blood bank), F - 44035 Nantes, Cedex 01, France

THE FIRST results for melanoma immunotherapy with TIL (tumour infiltrating lymphocytes), reported by Rosenberg and associates in 1988, showed an overall response rate of 55% [1]. Subsequent clinical studies gave results ranging from 17 to 35% [2–6]. However, the high intravenous IL-2 (interleukin-2) doses used in previous TIL immunotherapy protocols required hospitalisation, most often in intensive care units, because of severe cardiovascular toxicity.

The purpose of the present work was to assess the feasibility and tolerance of home adoptive immunotherapy in patients with stage IV melanoma who received TIL and IL-2 (the latter administered subcutaneously in low doses) in association with interferon-α (IFN-α). Moreover, for TIL production, a simpler, easier reproducible culture method was developed than that described in earlier studies.

6 patients were included in this study, all with stage IV malignant melanoma. Therapeutic targets were subcutaneous in 3 patients, pulmonary in 2, involved intra-abdominal nodes in 2, bone in 1 and mucous in 1. The characteristics of patients are summarised in Table 1. TIL were extracted from a nodal metastasis in 3 cases and from a subcutaneous metastasis in the other 3 cases. Our laboratory methodology used to produce TIL has previously been reported [7, 8]. Approximately 4 weeks before the first re-injection of TIL, a single cisplatin injection (100 mg/m^2) was performed for immunosuppressive purposes.

Administration of recombinant IL-2 (Proleukin*, Eurocetus*), begun the evening after TIL reinjection, consisted of a subcutaneous injection of 3.6×10^6 IU/m^2 per day, 5 days per week, for 2 weeks. Recombinant interferon-α2a (Roferon-A*, Roche*), started at the same time, consisted of a subcutaneous injection of 3×10^6 IU per day, 3 days per week, in continuous treatment. If cell expansion permitted, a second reinjection of TIL in association with IL-2 was performed 1 month later. Thus, each patient was hospitalised for only 24 h to receive TIL and initiate cytokine therapy. The following subcutaneous injections of cytokines were carried out at home by a nurse. The maintenance cycles consisted of continuous subcutaneous IL-2 doses (3.6×10^6 IU/m^2 per day), 5 days per week, 2 weeks per month. Treatment was stopped in case of progression or stabilisation at the end of the first maintenance cycle.

Among the 6 patients treated, there was one complete remission (CR) (subcutaneous metastases) lasting 4 months and 3 cases of >50% partial remission (PR) (subcutaneous, mucous metastases and pulmonary metastases) lasting 5, 5 and 7 months. No grade III or IV toxicity was noted during treatment. All patients were able to receive therapy at home with a good quality of life. The most common side-effects were erythematous subcutaneous nodules at the IL-2 injection site, which disappeared spontaneously within a few weeks, and a pseudo-flu syndrome.

Our culture method, involving short exposure to phytohaemagglutinin and the use of feeder cells (an EBV-transformed B line), enabled us to achieve sufficient TIL expansion *in vitro* (5 of the 6 patients received two reinjections, with a mean number of 17×10^9 cells) with moderate IL-2 doses (150 IU/ml), hopefully providing for more specific cytotoxic activity [7–9]. Analysis confirmed that only T lymphocytes were present in all cases since 100% of the cells were labelled by CD3. TIL were predominantly CD8 lymphocytes for patients 1 (PR) and 5 (progression), and CD4 for patients 2 (PR) and 4 (progression). Patient 3 (CR) had approximately equal proportions of CD4 and CD8, and for patient 6 (PR), the CD4/CD8 ratio differed between the first and second

Correspondence to B. Dréno
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*No specific mean.