**CELLULAR IMMUNE RESPONSES AGAINST MELANOMA CELLS.**

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Malignant melanoma is an important model for the study of cellular immune responses against cancer cells. We have isolated lymphocytes of normal donors in distinct fractions by centrifugal elutriation. Two fractions, small lymphocytes (SL) and large lymphocytes (LL), were found to differ in cytoytic activity against melanoma and other tumor cells. The LL appear to be more cytotoxic and this cytotoxicity can be enhanced by culturing with IL-2, whereas SL could hardly be stimulated by IL-2.

Cytotoxic T lymphocyte (CTL) clones can be derived from peripheral blood of melanoma patients by a mixed lymphocyte/tumor culture with irradiated autologous tumor cells and cloning in the presence of IL-2. The cytotoxic activity of these clones could be blocked by two monoclonal antibodies NKI-M6 and NKI-M7, produced in our laboratory. Biochemical analysis showed that NKI-M6 recognizes a proteoglycan consisting of a chondroitin sulfate component with a MW of 450 kD and a core protein of 250 kD. NKI-M7 recognizes the vitronectin receptor, a structure belonging to the integrin family that is involved in adhesion processes.

Recently we have obtained another series of T cell clones that are cytolytic to autologous melanoma cells after culturing with rIL-4 instead of rIL-2. It appeared that clones cultured with rIL-4 preferentially kill autologous melanoma cells as compared to K562, whereas clones cultured with rIL-2 kill K562 as well as autologous melanoma cells.

Analysis of the reactivity of the clones cultured in vitro may contribute to the knowledge of immunologically important structures on melanoma cells.

**ACTIVATION OF ONCOGENES IN HUMAN MELANOMA: A MESSAGE TO THE IMMUNE SYSTEM?**

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To study the activation of oncogenes in human melanoma we used two different approaches.

Firstly, mutations in the family of ras genes (H-, K-, and N-ras) were assayed by the polymerase chain reaction (PCR) method using oligonucleotide probes specific for the various mutations at positions 12, 13 and 61 of the p21 ras protein. We have investigated 46 fresh tumor specimens (9 primary tumors and 37 metastases) and 12 melanoma cell lines from 39 patients.

In the specimens of 7 patients, only mutations in the N-ras gene were found. No mutations could be detected in the other ras genes, indicating that in melanoma, the N-ras gene is prone to activation. In one patient with activated N-ras, both the primary tumor and the one metastasis contained the mutation and in another patient all 14 metastases were affected, indicating that the mutation is an early event in carcinogenesis. Since activated ras genes have been reported to be capable of altering the sensitivity of cells to Natural Killer (NK) cells, we assayed the lysis of three melanoma cell lines with activated N-ras. No significant differences between the NK lysis of these cell lines as compared to cell lines without N-ras activation were found, suggesting that in melanoma, ras activation does not contribute to the sensitivity of the tumor cells to NK cells.

Secondly, we analyzed the expression of 17 proto-oncogenes in the 12 melanoma cell lines at the level of mRNA on Northern blots. c-myc was the only oncogene with variable expression. Interestingly, an inverse correlation between c-myc expression and the expression of the Class I Major Histocompatibility Complex (Class I HLA) was found. Down-modulation of Class I HLA by c-myc could be established by transfection experiments using purified c-myc constructs.

Furthermore, we have found that regulation by c-myc is highly locus-specific and results in a strong down-modulation of the expression of HLA B, rather than A and C, in cells expressing high levels of c-myc. Since HLA Class I proteins...