CHAPTER IX

SUMMARY AND CONCLUSION
Chapter 9

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The metastatic spread of solid tumours is responsible directly or indirectly for most cancer-related deaths. Our understanding of the molecular genetic and biological events that contribute to tumour cell dissemination has increased considerably over the last decade. It is now clear that close anatomic and temporal co-operation between cellular adhesion molecules, extracellular matrix degrading enzymes and peptides inducing tumour vascularisation are essential components of the metastatic behaviour of cancer cells.

The formation of metastatic colonies is a continuous process that begins early in the growth of the primary tumour and increases with time (31,139,34). The main clinical significance of tumour metastasis is that it generalizes the disease process so that it can not be eradicated even by complete excision of the primary tumour. The important therapeutic modalities currently available for inhibition of metastasis include chemotherapy and immunotherapy in combination with other modalities of treatment like surgery or radiation. But all these treatment modalities have certain limitations. The search for a more effective treatment is still continuing and these include development of new drugs that can modulate immunity. Recently the use of plant products has emerged as an important therapeutic modality in cancer.

In the present study we have determined the effect of plant extracts and isolated ingredients for their effect in experimental metastasis and evaluated their potential use in immunotherapy. We have also tried to understand the mechanism of action of these drugs.
An extract of *Viscum album* has been reported to have significant antitumour activity not only in experimental animals but also in cancer patients. The effect of this drug in experimental metastases has been studied at present. Our laboratory has already established inhibitory effects of curcumin in experimental metastasis. Presently we have studied the immunological mechanism of action of curcumin and tried to correlate with its antimetastatic activity. Other plant extracts which were studied include *Santallum album*, *Piper longum* and *Glycyrrhiza glabra*. The immunopotentiating activity and their effect in experimental metastasis have been undertaken. Some of the salient conclusions of these studies are given below.

Administration of *Viscum album* extract (Iscador) simultaneously with tumour cells was found to produce maximum inhibition of tumour nodule (92%) formation, while administration of the drug either prophylactically (78.6%) or seven days after tumour induction (68.16%) produced reduction in the tumour nodule count. This was followed by a significant increase in the lifespan of animals.

Metastatic tumours produced significant biochemical changes in serum and tissues. For example, hydroxyproline which is a component of lung tissue collagen was increased in control tumour bearing lungs (29.9 μg/mg protein) indicating increased fibrosis of lung tissues. Iscador treatment reduced the level of lung collagen hydroxyproline (7.53μg/mg protein) almost similar to that of normal animals. Similarly it was found that tumour cells have changed surface properties from their normal counterparts and these changes are partially due to altered sialo-glycoconjugates expressed on the plasma membrane (16%). Hence sialic
there is increased expression of surface sialic acid on circulating tumour cells which facilitate their invasive behaviour. Control tumour bearing animals showed elevated level of sialic acid (147.6 µg/ml serum) compared to normal animals (23.6 µg/ml serum). The serum sialic acid level of Iscador treated animals was significantly reduced (20.6 µg/ml serum) and was similar to that of normal animals.

Invasion and adhesion are two major steps in the process of metastatic dissemination. Metastatic tumour cells disseminating through the blood must penetrate the capillary basement membrane and this extra cellular matrix is a mechanical barrier to invasion. For the tumour cells to carryout invasion it has to attach to the extracellular matrix through cell surface receptors that bind to specific adhesion molecules in the matrix. It is becoming clear that the interaction of invading tumour cells with the extracellular matrix is a complex process and probably involve a whole set of different receptors with distinct binding specificities. Iscador was found to inhibit the adhesion of B16F-10 melanoma cells to collagen matrix. Although we do not know the actual mechanism of action of Iscador in the inhibition of adhesion of tumour cells the following mechanisms are suggestive a) Iscador may produce a modification of cell surface receptors and hence invasion of the tumour cells was inhibited because of the altered cell surface properties. b) Inter cellular adhesion molecule (ICAM-1) was found to be increased in metastatic tumour bearing animals compared to normal animals. ICAM-1 is highly expressed on melanoma cells and its expression has been often associated with their malignant phenotype (167,169). Iscador treatment did not show any inhibition of collagenase activity as seen from the zymographic analysis.
Iscador was found to be useful in adoptive immunotherapy in metastatic tumour bearing animals. When spleen cells which were treated with Iscador was injected to tumour bearing animals there was a significant inhibition of lung tumour nodule formation which was 93.76% as compared with untreated tumour bearing animals (P<0.001). Average lifespan of these animals were also increased significantly.

Serum gamma glytamyyl transpeptidase which is a marker of cell proliferation was found to be increased in metastatic tumour bearing animals. Induction of γ-glutamyl transpeptidase has been reported during the growth of tumour cells (16%). Tumour bearing animals treated with splenocytes (activated with Iscador) showed reduced levels of γ-glutamyl transpeptidase levels thereby indicating the effectiveness of adoptive immunotherapy using Iscador.

Tumour bearing animals when depleted of natural killer cells using antiasialo GMI Ab the control animals showed massive lung tumour modules and elevated levels of lung tissue hydroxyproline and serum sialic acid levels. Tumour bearing animals depleted of natural killer cells when treated with splenocytes activated with Iscador showed reduction of lung tumour nodule formation.

It was found that cytokines such as IFN-γ and IL-2 were increased significantly in the serum of tumour bearing animals treated with Iscador. These cytokines are stimulators of natural killer cell activity. In metastatic tumour bearing animals Iscador treatment showed highly enhanced natural killer cell activity on 72h after tumour induction and was significantly (P<0.001) higher than controls. The antibody dependent cell mediated
immunity was also elevated in drug treated animals compared to tumour control. Enhancement of natural killer cell activity is an important activity shown by Iscador and is useful in the treatment of cancer cell metastasis. Natural killer cells are effectors of nonspecific nature and are active killers in the early stages of tumour spread. Serum tumour necrosis factor level was found to be lowered in Iscador treated tumour bearing animals compared to control tumour bearing animals. GM-CSF level was also found to be lowered in drug treated tumour bearing animals as compared with the control animals. It has been reported that in malignant conditions GM-CSF may promote metastasis by the production of IL-1 and TNF (155). Iscador was found to inhibit the levels of GM-CSF and INF-α. Inhibition of these cytokines may further inhibit the metastatic process.

In summary it could be infered that effective inhibition of metastasis may be due to

a) Production of cytokines-IL.2 & IFN-γ
b) Inhibition of TNF-α and GM-CSF
c) Subsequent activation of NK-cells.
d) Inhibition of adhesion of tumour cells to endothelial wall due to reduced ICAM-1 expression.

It should be added that Iscador is cytotoxic to many tumour cells and was nontoxic to cells of lymphoid origin. It was shown to inhibit the growth of many animal tumours and clinically highly effective. Hence the results presented in the study for the first time show that Iscador could inhibit metastasis and is highly significant in cancer therapeutic practice.
Myelosuppressive effects of radiation and chemotherapy are the main reasons for treatment failure in cancer patients. Administration of curcumin isolated from Curcuma longa was found to enhance the total WBC count, antibody titre and the number of plaque forming cells indicating possible immunopotentiating activity of curcumin. It was also found to enhance the spleen and thymus weight significantly (P<0.001) in normal as well as irradiated animals. Bone marrow cellularity and the number of α-esterase positive cells were also enhanced in curcumin treated animals after irradiation and chemotherapy which is a useful indication of its importance as an immunostimulating agent. Stem cell proliferation is an important property needed for the tumour surveillance and immune defense. Curcumin was found to cause enhanced proliferation of bone marrow stem cells, spleen cells and thymus cells in the presence of mitogens such as PHA and Con A.

GM-CSF was found to be increased in Curcumin treated animals which was found to be similar to normal animals. Interferon-γ and Interleukin-2 were also significantly (P<0.001) elevated in curcumin treated animals indicating the potency of curcumin as an immunomodulator. These two cytokines are potent stimulators of natural killer cells. Antibody dependent cell mediated cytotoxicity was also elevated in drug treated groups. IL-2 was found to promote the lymphokine mediated killer activity. As the tumour cells could be destroyed before the tumour load is too large, an early activation of NK cells is beneficial to the host during cancer metastasis. In fact the enhanced levels of IFN-γ and IL-2 was found in tumour bearing mice after curcumin treatment was correlated with increased natural killer cell activity. Untreated metastatic tumour bearing
animals showed elevated levels of tumour necrosis factor and GM-CSF which are promoters of metastasis whereas the drug treated group showed an inhibition of TNF production and lowered levels of GM-CSF. Curcumin could be used as an adjuvant for cancer treatment as it enhances the cytokine production. Natural killer cell activity was increased in Curcumin treated normal as well as tumour bearing animals.

The results of the present study indicated the effectiveness of Curcumin as a stimulator of both humoral and cellular immune responses. Since curcumin is nontoxic, without any side effects which could effectively stimulate the immunity and inhibit metastasis, curcumin could be used as an adjuvant in the cancer treatment. Extracts of the plants *Piper longum, Santallum album* and *Glycyrrhizha glabra* were also screened and found that their immunomodulatory activity was correlated with their antimetastatic properties. The use of immunomodulators of plant origin may be highly useful to circumvent the metastasis as well as to reduce the cancer recurrence.