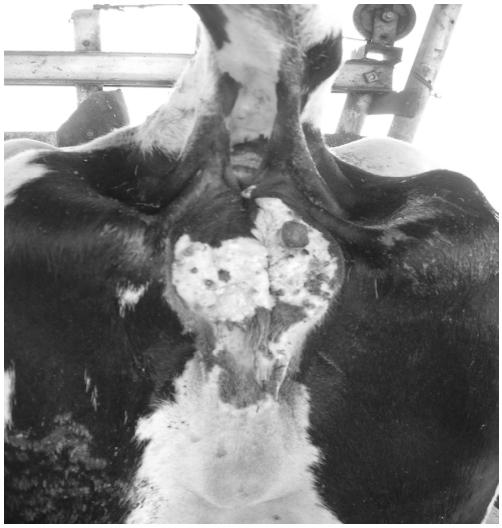
Chapter 1

General Introduction



Interleukin Therapy of Cancer

Oncotherapy for veterinary patients must be easy to use, readily available, reliable and cheap. The current options such as surgery, irradiation or chemotherapy are either not as effective as the animal owner would like or incur too much animal suffering and cost for the owners to bear. Successful alternative methods in animals could easily be applied to human cancer patients.

Methods for enhancing an animal's or human being's immune system to help make tumours regress have been used since the 19th century (Hall, 1997). The best documented clinical studies in this field were carried out by the New York physician William B Coley. He treated cancer patients with bacterial extracts known as "Coley's toxins". The studies were anecdotal cases but considering the medical knowledge of the day the success of Coley's treatments was most remarkable (Fiers, 1995). Stimulating the immune system as a method for oncotherapy has been used in veterinary patients. Spradbrow et al (1977) successfully induced tumour regression by a single intramuscular injection of the aqueous phase of a saline-phenol extract prepared from ocular squamous cell carcinomas collected from cattle, and they showed that the regression was associated with a cell- mediated immune reaction. Other groups have confirmed this finding (Hoffman et. 1981; Kainer et al. 1984), and other studies have shown that peri- or intra-tumoural injections of bacillus Calmette-Guerin (BCG) can cause BOSCC to regress (Kleinschuster et al. 1977, 1981, 1983; Klein et al. 1982, 1986, 1991; Rutten et al. 1991a).

Cytokine therapy of cancer is an increasingly used method of treatment. Cytokines are physiologically active in minute concentrations. Cytokines in low concentrations are far less cytotoxic than chemotherapy and therefore result in fewer adverse side effects.

Generally systemic treatment is used in human cancer patients with some systemic toxic effects. Rosenberg et al. combined high systemic doses of IL-2 with the systemic infusion of lymphokine – activated killer cells (LAK cells) for the treatment of human cancer patients. This treatment mode resulted in high levels of adverse side effects and only up to 10% of patients showing a complete remission (Rosenberg et al. 1987, 1990).

In comparison Den Otter and numerous other researchers and groups have performed numerous studies on the positive effect of local IL2 and IL12 therapy of cancers in various animal models, veterinary tumours and human tumours, with minimal side effects and much higher remission rates (Den Otter et al. 1991, 1995a, 1998, 1999; Forni et al. 1986; Kaplan et al. 2000; Maas et al. 1991; Masztalerz et al. 2003; Roth et al. 1989; Rutten et al 1989, 1991b; Spoormakers et al. 2003; Tubaro et al 1995). This thesis is a continuation of those studies.

Bovine ocular squamous cell carcinomas (BOSCC) and Bovine vulval papilloma carcinoma complex (BVPCC) are two spontaneously occurring tumour models that due to their growth sites are amenable to local IL2 and IL12 therapy trials. Both tumours are highly prevalent in Bos taurus breeds of cattle found on dairy and beef farms in Zimbabwe, including Holstein Friesian, Hereford and Simmental cattle as well as their crosses. BOSCC and BVPCC are economically important as they can both result in life threatening carcinomas, metastases and

loss of valuable cattle, milk, calves and slaughter carcasses. They are important as a model for potential cytokine therapy of human cancer as they are spontaneously occurring and other modalities of therapy are available for comparison of efficacy. In addition progression of both diseases is well documented.

Other reasons for selecting these tumours for this thesis were: the clinical diagnosis is reliable and the outcome of untreated tumours is highly predictable; the lesions can be closely monitored, reliably and easily; biopsies can be taken relatively easily; they are the most common tumours in cattle, and in Zimbabwe there are large numbers of affected cattle available for study.

Bovine Ocular Squamous Cell Carcinoma

Bovine Ocular Squamous Cell Carcinoma (BOSCC) is one of the most common disease conditions of eyes of cattle around the world (Cordy 1990). It is probably the most common cancer of cattle (Bastianello 1992; Cordy 1990; Hamir and Parry 1980; Heeney and Valli 1985) . It is very common in white-faced breeds of cattle or cattle with unpigmented skin around the face and in particular the eyes. It most commonly affects Hereford, Simmental, Holstein Friesian and Ayrshire breeds of cattle as well as their crosses (Cordy 1990; Den Otter et al. 1995b; Heeney and Valli 1985; Russell at al. 1976; Sloss et al. 1986). Increased prevalence of BOSCC is associated with increased annual hours of sunshine, increased altitude, and decreased latitude. Anderson et al (1991) found that the average ages of affected cattle tended to be lower at high radiation levels than at low levels.

Susceptibility to BOSCC is known to be heritable in Hereford cattle. Heritability estimates vary widely, but range from 17 to 66 percent (Cordy 1990; Russell et al 1976; Sloss et al 1986). The peak age for BOSCC is between 7 and 8 years of age, although the condition has been reported to occur in cattle under the age of 3 years (Cordy 1990; Den Otter et al 1995b).

Other factors associated with BOSCC have been examined. A relationship between a high level of nutrition and an increase in BOSCC has been demonstrated in Hereford cows. Viruses have also been associated with the disease but not proven to be a cause. Infectious bovine keratoconjunctivitis (pink eye) has not been shown to be involved. The tumours occur at an equal rate in males and females, although an artificially higher rate is reported in females, as they are kept in larger numbers and for longer in breeding herds (Cordy 1990).

BOSCC can occur on a number of different sites of the bovine eye. According to some literature the most common site (83 percent) is the limbus, which is the junction of the cornea and the sclera. Sixty-seven percent occur on the lateral limbus and 16 percent on the medial limbus. The remaining 17 percent occur on the eyelids, including the third eyelid (Cordy 1990). Other authors state that the most common site is the third eyelid, followed by the limbus, lower eyelid and canthus (Den Otter et al. 1995b; Klein et al 1986; Stewart et al 2006). Nonpigmented regions of the eye are more predisposed to BOSCC because of reduced protection from

ultraviolet sunlight (Cordy 1990; Anderson et al 1991). Growths on the cornea are less prone to metastasize than tumours on the sclera (Cordy 1990).

There are four stages of development for BOSCC. These include plaques (stage 1); keratoma, or keratoacanthomas (stage 2); papillomas (stage 3); and carcinomas (stage 4). Plaques appear as small, white, elevated areas. Keratomas occur more frequently on the lower eyelid. They are skin growths coated with eye secretions and debris. Papillomas may have a wart-like appearance. Carcinomas are more irregular and nodular and may have a pink colour due to an increased blood supply (Cordy 1990; Den Otter et al 1995b). Plaques, keratomas, and papillomas (stages 1, 2, and 3) are benign. Carcinomas (stage 4) are malignant (Cordy 1990).

There are a number of treatment options available for BOSCC. The most common treatment method is surgery which involves either removal of just the tumour or enucleation (removal of the affected eye). In herds where large numbers of animals are affected this is not a feasible option so large numbers of animals are culled due to the tumour. Local injection of interleukin 2 and/ or 12 is a more feasible option, especially since some studies have now found that a single injection is equally as effective as multiple injections (Den Otter et al 1999)

Bovine Vulval Papilloma Carcinoma Complex

BVPCC is a common disease in exotic breeds of cattle kept at high altitude in Africa, with authors finding prevalences ranging from 4% to 30% (Burdin 1964; Hill et al 1994; Stewart personal findings). Histopathological staging of BVPCC has been described by Burdin (1964) and adapted by Hill et al. (1994) for clinical staging as follows:

Stage 1: generalized thickening, corrugation and roughening of unpigmented vulval skin.

Stage 2: papilloma precursor – slightly protruding smooth white hyperplastic foci (up to 6 x 3 mm).

Stage 3: small size papillomas (3 – 6 mm at the base and up to 3 mm in length) showing some keratinization.

Stage 4: large size papillomas (3 – 15 mm at the base and a length of up to 100 mm including the keratinized terminal portion).

Stage 5: carcinomas; ulcerating masses with haemorrhagic surfaces with a diameter of up to 50 mm.

Stage 6: most of tumour deep and fistulated; vulval outline obliterated; Stages 1, 2, 3 and 4 frequently interspersed.

I have mostly seen BVPCC in Holstein Friesian cattle kept for dairying purposes in Zimbabwe. In these cattle neither, one or both vulval lips are pigmented or partly pigmented. I saw BVPCC lesions only on the unpigmented parts of the vulval lip even when parts of the same vulval lip are pigmented. I have never observed lesions on pigmented vulval lips or parts of vulval lips. This would suggest that part of the pathogenesis of BVPCC is related to exposure to UV radiation. Little information is available on BVPCC. However I have seen very high prevalence of BVPCC in cattle in Zimbabwe with up to 30 % of some herds affected. This may either be

due to the popular theory of a virus being involved in the pathogenesis or maybe to do with the genetics of vulval pigmentation in certain herds. Normally lesions are stage 3 or 4; however cows with severe life threatening carcinomas can be seen. This can lead to difficulty with calving and possibly death of the cow.

At present there are no treatment options available for BVPCC. Generally farmers keep the cows until the tumour interferes with the reproductive ability of the cows and then they cull them. Therefore an easily applicable treatment method that could prolong the reproductive life and ease the suffering of the animal would be welcomed.

Aim of this Study

The aim of this study is to determine the efficacy of various IL-2 and IL-12 therapy protocols in the treatment of BOSCC and BVPCC and to study the histology of the response to local IL- 2 and IL-12 therapy. We investigated:

- Efficacy of various therapeutic protocols for local application of IL2 and/ or IL12 in the treatment of BOSCC. (Chapter 2 and 3)
- Efficacy of various therapeutic protocols for local application of IL2 and/ or IL12 in the treatment of BVPCC. (Chapter 4)
- Histological studies of response to local application of IL2 and/ or IL12 therapy of both BOSCC and BVPCC post treatment. (Chapter 5)

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