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Harri Salo

Effects of Ultraviolet Radiation on the Immune System of Fish

UNIVERSITY OF JYVÄSKYLÄ

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ABSTRACT

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Diss.

There is a widespread concern over the increasing amounts of ultraviolet radiation reaching the earth due to stratospheric ozone depletion. Ozone depletion was first recognised as a problem in the middle of the 1980s and is estimated to continue until 2050. Ultraviolet radiation has harmful effects on human and animal health, including suppression of the immune system. Since ultraviolet radiation penetrates into water it has negative effects in fish species as well.

Adult roach, *Rutilus rutilus* cyprinidae, were exposed to a single dose of either ultraviolet A (UVA; 3.6 J/cm^2) or ultraviolet B (UVB; 0.5 J/cm^2) irradiation in order to study the effects of ultraviolet radiation on the immune defence of fish. A panel of immune parameters were assayed during a 14-day follow-up. Exposure to either UVA or UVB irradiation suppressed mitogen-stimulated proliferation of blood and splenic lymphocytes, but the production of antibodies was not affected. The activity of natural cytotoxic cells was suppressed following both types of irradiation. UVB, but not UVA, suppressed transiently the migration and respiratory burst of head kidney phagocytes. UVB exposure induced marked granulocytosis and lymphocytopenia in the blood and raised the plasma cortisol concentration, which suggests that stress induced by UVB is involved in the modulation of immune parameters. These results imply that both UVA and UVB have the potential to suppress nonspecific as well as specific aspects of the immune defence of fish.

Key words: Fish immune system; immunotoxicity; ultraviolet radiation.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals I-IV.

I Harri Salo, Tuula Aaltonen, Eveliina Markkula & Ilmari Jokinen (1998). Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae) I: Phagocytes. - Photochem. Photobiol. 67: 433 - 437

II Harri Salo, Ilmari Jokinen, Eveliina Markkula & Tuula Aaltonen (2000). Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae) II: Blood. - Photochem. Photobiol. 71: 65 - 70

III Ilmari Jokinen, Harri Salo, Eveliina Markkula, Anu Immonen & Tuula Aaltonen (2000). Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae) III: Lymphocytes. - Photochem. Photobiol. (revised and submitted)

IV Harri Salo, Ilmari Jokinen, Eveliina Markkula, Tuula Aaltonen & Heikki Penttilä (2000). Comparative effects of UVA and UVB irradiation on the immune system of fish. - J. Photochem. Photobiol. B: Biol. 56: 154-162

In addition, some unpublished data are presented.

ABBREVIATIONS

ASC	antibody-secreting cell
ACTH	adrenocorticotropic hormone
BGG	bovine γ-globulin
BSA	bovine serum albumin
CL	chemiluminescence
ConA	concanavalin A
CHS	contact hypersensitivity
DTH	delayed-type hypersensitivity
EGC	eosinophilic granular cell
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immuno spot
HPA	hypothalamic-pituitary-adrenal cortex axis
IFN	interferon
Ig	immunoglobulin
IL	interleukin
ISC	immunoglobulin-secreting cell
LC	Langerhans cell
LPS	lipopolysaccharide
MSH	melanocyte stimulating hormones
NCC	natural cytotoxic cell
NK	natural killer
PG	prostaglandin
PMA	phorbol 12-myristate 13-acetate
PMN	polymorphonuclear leukocyte
POMC	proopiomelanocortin
ROS	reactive oxygen species
Th	T helper cell
Th1	T helper cell subpopulation 1
Th2	T helper cell subpopulation 2
TNF	tumour necrosis factor
UCA	urocanic acid
UVA	320-400 nm radiation
UVB	280-320 nm radiation
UVC	100-280 nm radiation
UVR	ultraviolet radiation

1 INTRODUCTION

Sunlight is the most potent environmental agent influencing life on the earth. Historically, exposure to the sun has been believed to be healthful and beneficial. The sun's energy reaching the earth is, of course, the ultimate source of life, but it has only recently become apparent that many of the effects of solar radiation are detrimental. In a broad sense, therefore, the evolution of life can be regarded as a continuous adaptation to light by simultaneously utilising solar energy and protecting against its detrimental effects.

There is mounting concern over the increase in UVB radiation in the environment induced by ozone depletion. The impact of increased UVR on human health has been much studied but relatively little attention has been paid to possible detrimental effects on the health of terrestrial or aquatic animals. Experimental studies on animals indicate that exposure to UVR elicits transient and long-lasting effects in the skin and the eye, the severity of which increases in proportion to the level of exposure. One of the more harmful effects of UVR exposure in humans and animals is the interaction of UVR with the immune system and subsequent decrease in resistance to disease (Mayer 1992). Any increase in disease in, for example, domestic species would not only have serious animal welfare implications but could also be economically significant. Aquaculture systems which often have little or no protection afforded by shade may also be at risk. Cataracts and skin lesions have been associated with the exposure of farmed fish to ultraviolet radiation and have resulted in significant losses (Bullock 1988).

Toxicological studies in mammals have shown that the immune system is sensitive to the effects of pollution (Vos & van Loveren 1996). During recent decades the possible influence of environmental pollution on the aquatic ecosystems has attracted considerable interest. Fish, especially, have become a favoured subject of research. It has been indicated that certain immunological parameters can be used as suitable biomarkers for monitoring the impact of hazardous environmental factors (Wester et al. 1994).

The present study was planned to investigate the effects of ultraviolet radiation on the immune defence of fish. Interest was paid to the functioning of the different parts of the fish immune system and its modulation by both UVA and UVB radiation.

2 REVIEW OF THE LITERATURE

2.1 Solar ultraviolet radiation

The optical radiation emitted by the sun consists of ultraviolet radiation (UVR), visible light, and infrared radiation. UVR is shorter in wavelength and more energetic than visible light. UVR is more or less arbitrarily divided in three regions: ultraviolet C (UVC, 200 - 280 nm), ultraviolet B (UVB, 280 - 320 nm) and ultraviolet A (UVA, 320 - 400 nm).

2.1.1 Attenuation of UVR in atmosphere

UVR constitutes 8.3 % of solar irradiance prior to its attenuation by the earth's atmosphere (Frederick et al. 1989). Accurate characterisation of environmental UVR at the earth's surface is difficult because of large geographical and temporal variations such as earth-sun geometric factors, ozone amounts, cloudiness, various local pollutants, and reflectivity. Annual doses of UVR are maximal in the equatorial region, where they are, for example, five-fold greater than in Finland (Madronich et al. 1995).

Solar UVR undergoes significant absorption by the atmosphere. The most hazardous region of UVR, i.e. UVC, is absorbed in the upper atmosphere by molecular oxygen and ozone. UVB, however, is only partially attenuated by ozone and is therefore vulnerable to changes in stratospheric ozone levels, while UVA passes almost unhindered through the upper atmosphere (Madronich et al. 1995).

Long-term data series on the incidence of solar UVB radiation at the earth's surface indicate that over the past 10-15 years UVB levels have increased and that these increases are linked to reductions in stratospheric ozone (Crutzen 1992; Kerr & McElroy 1993; Madronich et al. 1995; Taalas et al. 1996). Although of lesser amplitude than in the Antarctic, reductions in the thickness of the ozone layer have also been detected over the Arctic (Goutail et al. 1999) and, as a result of air mass mixing, also at middle latitudes (Björn et al. 1998). Thus, ozone layer depletion with concomitant increases in UVB is a world-wide

phenomenon. However, it has been estimated that the latitudes above 40° north or south will receive the greatest increase in UVR (Coldiron 1996) and that the enhanced UVR doses may exist until at least the middle of the 21st century, particularly in the high latitude Northern Hemisphere (Taalas et al. 2000).

2.1.2 Absorption of UVR in water

Biologically effective doses of UVR penetrate into a water column (Smith & Baker 1979; Booth & Morrow 1997). The optical quality of the water is an important factor in the penetration of water by UVR. The depth of water required to remove 90% of solar radiation at 310 nm varies from about 20 m in the clearest ocean water to a few cm in brown humic lakes and rivers (Kirk 1994; Huovinen et al. 2000). As in the case of atmospheric penetration, UVA also has greater penetration in a water column than UVB radiation (Piazena & Häder 1994; Kuhn et al. 1999; Huovinen et al. 2000).

2.2 General effects of UVR on humans and animals

2.2.1 Humans and terrestrial animals

The cutaneous effects of ultraviolet radiation are a function of the penetration and absorption of particular wavelengths. In human skin UVB is absorbed predominantly by the stratum corneum, followed by absorption in the epidermis. Only a small fraction of UVB reaches the dermis (Slominski & Pawelek 1998). UVA has a greater skin penetration than UVB (Bruls et al. 1984).

UVB is strongly absorbed by DNA and certain other biomolecules of the skin, such as membranes and proteins (Kohen et al. 1995), and results in significant biological effects such as the synthesis of vitamin D3 (Adams et al. 1982). The biological effectiveness of UVA is markedly dependent on the presence of sensitisers, molecules which absorb UVR, and which indirectly result in biological damage, often via the production of active oxygen species (Tyrrell 1991). Indeed, it has been estimated that UVA is about 1000 times less biologically active than UVB (Saunders et al. 1997).

Acute responses of skin to UVR may be broadly divided into inflammatory, reparative and protective responses (Gange & Parrish 1983), and these have been seen in human and animal skin alike (Slominski & Mihm 1996). The initial phase of damage results in inflammation, with the classic signs of redness (erythema), swelling (oedema) and pain. This is followed by repair at the molecular and cellular levels, and then by adaptive changes such as thickening of the epidermis and stratum corneum and increased melanisation (tanning), which confer protection against subsequent irradiation.

The most serious detrimental effects on human health for which exposure to UVR is a recognised risk factor are the cutaneous malignancies. Skin cancers

can be divided into two main types, non-melanoma skin cancers (basal and squamous cell carcinomas) and malignant melanoma. The findings from epidemiological studies indicate that the risk of non-melanoma skin cancers is related to cumulative UVR exposure. Malignant melanoma is the main cause of death from skin cancer, although it is much less common than the non-melanoma type. The development of melanoma have been associated with intense, intermittent exposure to solar radiation. Perhaps the best skin cancer action spectrum has been developed for the squamous cell carcinoma in the hairless mouse (de Gruijl et al. 1993). This demonstrates that UVB is a major carcinogen, but is not to minimise the effects of UVA.

UVR also has effects on the immune system of human and animals. Suppression of a class of lymphocyte-mediated responses, which are important in combating certain types of infections and cancers, is regarded as a major effect of UVR on the immune system. The immunosuppressive effects are discussed in more detail in paragraph 2.3.

In addition to the skin, the eyes are also vulnerable to the harmful actions of UVR (Zigman 1993). Eyes have evolved to detect light. Some animal species can see UVR as a distinct colour, but the human eye filters out most of the radiation below 400 nm before it reaches the receptors. UVR can damage the cornea and lens of the human eye (Saunders et al. 1997). Well-known short-term ocular effects of UVR are photokeratitis and photoconjunctivitis, commonly known as snow-blindness or welder's eye. In cattle, UVR is considered to increase the susceptibility of the cornea to infection (Mayer 1992). Chronic exposure to UVB is associated with the risk of pterygium, an outgrowth of the conjunctiva over the cornea, and cataracts, the loss of transparency of the lens.

2.2.2 Aquatic environment: fishes

Fish skin is an delicate membrane, and differs from its mammalian counterpart in many respects. The outermost layer, the cuticle, is acellular and very vulnerable to damage. In the fish epidermis, the malpighian cells are the fundamental cellular component and thus comparable to the keratinocytes in human skin. Malpighian cells are able to divide in all layers of the epidermis and they do not contain keratin. This indicates that fish skin is naturally vulnerable to UVR and when compared to the skin of terrestrial animals which is often shielded from solar radiation by fur.

The existence of DNA damage in the UVB-exposed fish skin has been well demonstrated (Ahmed & Setlow 1993; Mitani et al. 1996; Vetter et al. 1999). Furthermore, UVB radiation induces sunburn (i.e. darkening of the skin), the appearance of sunburn cells and depletion of the mucus layer and, in more severe cases, even sloughing of the epidermis in exposed skin sites (Bullock 1988; Fabacher et al. 1994; Blazer et al. 1997).

In several fish species melanocytes lie in the dermis and do not give pigmentary protection to epidermal cells (Bullock 1982). UVR-inducible

pigments or substances, however, give protection to cells in the epidermis in some fish species (Hunter et al. 1979; Fabacher & Little 1996; Lowe & Goodman-Lowe 1996). Epidermal hyperplasia is also a protective response of fish to UVR (Bullock 1988). Reparative responses to the UVR-induced damage in fish includes, for example, DNA repair mechanisms such as efficient photolyase activity, which is inducible by UVR and blue light, (Ahmed et al. 1993; Funayama et al. 1996; Mitani et al. 1996).

UVR-induced injuries of the skin may be accompanied by infections (Fabacher & Little 1996) and can lead to increased mortality. Losses of several fish species due to overexposure to solar or artificial UVR have been documented (Hunter et al. 1982; Bullock 1988; Fabacher et al. 1994; Little & Fabacher 1994; Beland et al. 1999; Kouwenberg et al. 1999a; Kouwenberg et al. 1999b). A majority of the studies concerning the detrimental effects of UVR on fish emphasis the effectiveness of UVB, while the role of UVA has been depicted as deleterious (Winckler & Fidhiany 1996; Bass & Sistrun 1997; Williamson et al. 1997), non effective (Kouwenberg et al. 1999a; Kouwenberg et al. 1999b) or protective (Mitani et al. 1996).

UVR can also affect the eyes of fish. High doses of UVB can produce corneal damage as well as cataractous changes on the eyes of rainbow trout (Doughty et al. 1997). The incidence of cataract in salmon had increased from negligible levels to 55% within ten years (1979-1989) off the West coast of Scotland. However, during this period a chemical (Nuvan 500 EC) was being used in salmon farming to control sea-lice and it may have interfered with the normal eye protective mechanisms, thus possibly rendering UV-exposures more hazardous still (Frazer et al. 1990).

2.3 Photoimmunology

All higher organisms require an intact immune system to detect and destroy invading micro-organisms (bacteria, fungi, viruses and parasites) and to eliminate cells that have undergone malignant transformation.

The skin is anatomically the first barrier to microbe invasion. It contains elements of the immune system and is accessible to UVR. Immunocompetent skin cells are either of resident (for example keratinocytes, endothelial cells), recruited (monocytes, granulocytes) or recirculating type (T lymphocytes, dendritic cells), and together they form the skin immune system (Bos et al. 1997).

2.3.1 Immunological outcomes of UVR exposure

Over the last two decades it has became clear that UVB exposure can impair specific and nonspecific immune responses. UVB-induced impairment of immunological functions are associated with the induction of skin cancers

(Kripke 1981) and reduced resistance against viral, fungal and bacterial infections as well as against parasitic diseases (Jeevan & Kripke 1993; Hurks et al. 1994; Grabbe & Granstein 1995; Garssen et al. 1998b). It is noteworthy that UVR effects are not restricted to skin-associated infections but are also associated with systemic infections.

On the basis of their cytokine profiles, T helper (Th) cells, which orchestrate the immune defence responses, can be subdivided into two major subsets of Th effector-cell populations. The T helper 1 (Th1) subpopulation produces interleukin-2 (IL-2) and interferon-γ (IFN-γ), promotes delayed-type hypersensitivity (DTH) responses, activates macrophages, may be particularly important in dealing with antigens expressed on cell surfaces, such as viral and tumour antigens, and provides help for certain antibody isotype responses, including complement fixing antibodies (Coffman et al. 1988). Today the evidence that the Th1-augmented immune branch is suppressed by exposure to UVB is quite firm. For example, contact hypersensitivity (CHS) and DTH responses are suppressed following UVB exposure in exposed (local) and nonexposed (distant) skin areas (De Fabo & Noonan 1990). Suppressed DTH-like responses following UVR exposure have also recently been demonstrated in bronchus (systemic suppression) of mice (van Loveren et al. 2000). In contrast, T helper 2 (Th2) cells produce IL-4, IL-5, IL-6 and IL-10, and promote antibody responses. Only Th2 cells can stimulate a primary IgE response which is mediated by IL-4 and inhibited by IFNγ (Coffman et al. 1988). Earlier studies have concluded that Th2 cell responses are not affected or are even enhanced following UVB exposure (Araneo et al. 1989), but recent studies in rodent models have indicated that Th2-mediated responses, for example antibody production, can also be suppressed following exposure to UVB (Goettsch et al. 1996a; Goettsch et al. 1996b; Garssen et al. 1999; van Loveren et al. 2000).

In addition to the above-mentioned suppression of specific, lymphocyte-mediated immune defence mechanisms, UVB can also disturb nonspecific cellular immune defence mechanisms. Exposure to UVB causes reduced cytotoxic activity of natural killer (NK) cells in rats and humans (Hersey et al. 1988; Goettsch et al. 1996b). In mice, phagocytosis by intraperitoneal or splenic macrophages is suppressed following UVB exposure (Jeevan et al. 1995). The few studies concerning the activity of blood phagocytes are contradictory. In studies where UVB is the main component of exposure down-modulated activities have been described (Lundin et al. 1990; Goettsch et al. 1996b; Leino et al. 1999), but exposures in which UVA dominates up-modulated phagocyte activities have also been seen (Csato et al. 1984; Meffert et al. 1989; Müller et al. 1990; Meffert & Scherf 1992).

UVB changes the distribution of the cells of the immune system. In response to UVB, epidermal Langerhans cells (LCs) lose their dendritic appearance and capacity to present antigens, and leave the skin (Cooper et al. 1992). Endothelial cell enlargement and occasional massive venular dilation have been noted following UVB and UVA irradiation (Gilchrest et al. 1981; Gilchrest et al. 1983), and, together with increased expression of endothelial

leukocyte adhesion molecules (Norris et al. 1991; Heckmann et al. 1994), allows the infiltration of T lymphocytes into perivascular spaces (Gilchrest et al. 1983; Di Nuzzo et al. 2000). Similarly, the migration of antigen-presenting macrophages and inflammatory neutrophils into the exposed area are increased (Gilchrest et al. 1983; Hawk et al. 1988; Cooper et al. 1993). Lymphocytes also accumulate in the peripheral lymph nodes (Spangrude et al. 1983). UVB radiation increases the number of granulocytes and decreases the number of lymphocytes in the human circulation (Hersey et al. 1983; Gahring et al. 1984; Lundin et al. 1990) and in several mammalian species (Kosar 1974; Spode 1974; Ebbesen 1981; Flindt-Hansen & Ebbesen 1991; Goettsch et al. 1994a).

Exposure to UVA radiation also causes immunomodulation in mammals (Krutmann 1995), although its effects are currently less well-defined and more controversial than those of UVB radiation. For example, mouse CHS reactions to topically applied haptens are suppressed (Halliday et al. 1998; Iwai et al. 1999) or not affected (Laihia & Jansen 1994; Skov et al. 1997; Reeve et al. 1998) following exposure to UVA. However, evidence exists that LCs (Baadsgaard et al. 1989; Iwai et al. 1999), T lymphocytes (Morison et al. 1979), NK cells (Hersey et al. 1988), mast cells and dermal endothelial cells (Heckmann et al. 1994) are affected by UVA radiation.

2.3.2 Immunomodulating mechanisms induced by UVR

DNA and urocanic acid (UCA) are skin chromophores that have been demonstrated to mediate the effects of UVR on immunity. Wavelengths in both the UVB and UVA regions can induce both DNA damage in exposed cells (Roza et al. 1989) and the photoisomerisation of *trans*-UCA to *cis*-UCA in the stratum corneum (Kammeyer et al. 1995). Certain other biomolecules, such as proteins or membranes, are also involved in the absorption of UVR photons (Tyrrell 1994; Schwarz 1998), and may mediate physiological changes by causing, for example, lipid peroxidation or induction of reactive oxygen species.

Exposure to UVR induces secretion of cytokines by epidermal cells (Noonan & De Fabo 1992; Berneburg & Krutmann 2000). Keratinocytes are the outermost living cells of the human skin, and thus are readily exposed to UVR. Upon UVB irradiation, keratinocytes produce cytokines such as interleukins (IL-1, IL-6, IL-8, IL-10), growth hormones such as granulocyte macrophage colony stimulating factor, tumour necrosis factor α (TNFα), eicosanoids, and neuropeptides such as proopiomelanocortin (POMC) and its derivatives (Beissert & Granstein 1997; Luger 1998). Moreover UVA induces epidermal keratinocytes (Aubin et al. 1991; Krutmann 1995), and also, due to the deeper penetration into the skin than UVB, dermal fibroblasts (Wlaschek et al. 1993) to produce immunomodulatory cytokines.

Cis-UCA can be detected in the blood and urine following UVB exposure, indicating that UCA may be involved in the systemic effects of UVB (Moodycliffe et al. 1993; Kammeyer et al. 1994). Some of the UVR-inducible

cytokines, such as IL-1, IL-6 and TNF-α, are also systemic and may be relevant in the pathogenesis of systemic sunburn reaction, which is characterised by fever, leukocytosis and the induction of acute phase response, and plays an important role in the mediation of immunological and inflammatory reactions (Beissert & Granstein 1997). On the other hand, eicosanoids formed in UVB-exposed skin are relatively short-lived and act only locally (Beissert & Granstein 1997).

It has been postulated that *cis*-UCA formed in the epidermis causes the dermal mast cells to release histamine, which mediates an early phase of the human sunburn reaction (Gilchrest et al. 1981) and participates also in UVR induced immunomodulation (Hart et al. 2000).

Nerves in the skin have the potential to modulate skin immune responses (for review, see Downing and Miyan, 2000). Certain neuropeptides, such as calcitonin gene-related peptide (CGRP), are involved in cutaneous inflammation as well as in systemic immunosuppression following UVB exposure (Benrath et al. 1995; Garssen et al. 1998a). POMC-peptides are released from the pituitary gland under IL-1 stimulation (Luger & Schwarz 1995), but also in response to UVB from the keratinocytes (Luger 1998). In mammals POMC-peptides are involved in the stress response via the hypothalamic pituitary adrenal cortex (HPA) axis. Neuroendocrinology and immunology are firmly linked to each other and can be considered a potential mechanism in UVB-induced immunosuppression (Luger 1998).

2.4 Fish immune system

Jawed fishes developed nearly 400 million years ago during the Silurian period. Their descendants, the bony fishes (Osteichthyes) and cartilaginous fishes (Chondrichthyes) are the predominant fish forms in present day seas and freshwater. Over 20 000 different fish species currently exist and their immune systems appear to be quite varied.

The immune system in teleost fish (reviewed by e.g. Anderson and Zeeman, 1995; Iwama and Nakanishi, 1996; Nakanishi, 1999) is highly evolved and functions to provide the organism with the ability to resist infectious agents, destroy neoplastic cells, and reject nonself components. The fish immune system is in many respects comparable to the mammalian immune system. For example, the ability of fish leukocytes to phagocytize and kill foreign pathogens, produce specific antibodies, reject allografts, secrete cytokines, and many other responses are shared with the mammalian immune system. However, there are also differences between these two systems. One of the most striking differences is the absence of bone marrow and lymph nodes. In fish, functionally equivalent hematopoietic tissues to mammalian bone marrow are found in areas of the spleen, head kidney and thymus (Zelikoff 1994). Antigen presentation, similar to that in the germinal centres of

mammalian lymph nodes, is thought to occur in melanomacrophage centres, which are scattered throughout the body, although concentrated in the spleen, liver and kidney (Lamers et al. 1985; Press et al. 1994). However, this view is not acknowledged by all authors (Zapata et al. 1996).

The first line of defence by fish against microbes is represented by the epithelia covering gills, skin, and gut. These epithelia secrete a layer of mucus that contains humoral defence factors such as antibodies, complement, and lysozyme produced by cells of the immune system (Shephard 1994).

The immune system of vertebrate animals is commonly divided into two functional defence entities: the nonspecific (innate) and the specific (acquired). The nonspecific pathway is a first defence mechanism following trauma or invasion by foreign pathogens. It does not require prior contact with the pathogen and lacks specificity. A variety of leukocyte types are involved in the nonspecific cellular defences of fish. Macrophages, monocytes and granulocytes are mobile phagocytic cells important in inflammation, which is the cellular response to microbial invasion and/or tissue injury. These cells possess destructive enzymes and are able to produce reactive oxygen radicals that can destroy pathogens. Less mobile tissue granulocytes, eosinophilic granular cells (EGCs), are also involved in the host response to bacterial and helminth pathogens. They are found in gills, gut, skin and around major blood vessels and are reminiscent of mammalian mast cells because they can degranulate and release immunopharmacological agents, such as histamine, in a manner analogous to mammalian mast cells (Vallejo & Ellis 1989). Virus-infected host cells and protozoan pathogens may be the target for natural cytotoxic cells (NCCs), which are considered to be equivalent functionally to mammalian NK cells (Evans & Jaso-Friedmann 1992). NCCs can spontaneously kill foreign cells. Nonspecific humoral factors, such as complement, lysozyme, interferon, lectins etc., are also of importance (Iwama & Nakanishi 1996).

Specific immune responses are directed against an agent to which the organism has previously been sensitised. It is well established that fish possess lymphocyte populations analogous to T and B cells, and thus both cellular and humoral specific responses exist. Cell-mediated immunity (reviewed by Nakanishi, 1999) involves e.g. the generation of cytotoxic T-lymphocytes against intracellular viruses and the cytokine mediated modulation of immune responses (Secombes et al. 1999). The humoral branch of the specific immune system involves the production of immunoglobulins by activated B cells (plasma cells). Immunoglobulins help in antimicrobial defence by neutralising viruses in the fluid compartments, opsonisation of microbes and aiding the complement-mediated lysis of microbes. The major immunoglobulin class in teleosts is IgM (Kaattari & Piganelli 1996).

2.4.1 Factors regulating the fish immune system

The immune system of fish, as one aspect of their physiology, can be affected by many of the environmental and internal factors they encounter (Reviewed by

Tatner, 1996). During ontogeny there is a sequential development of lymphoid organs and immune responses, with nonspecific immunity developing first, followed by cell-mediated and then humoral immunity. Also, sexual maturation and breeding have effects on the fish immune system both by the action of hormones and as a consequence of the reduced allocation of energy resources to the immune system during periods of breeding. On the other hand, there are natural external factors that can affect immune functions, such as diet, temperature, time of the year etc. Fish live closely with their aquatic environment since living epidermal cells are in direct contact with all substances in the water. This intimate contact facilitates the movement of chemicals into and through the mucus and skin, and becomes a disadvantage to the fish when harmful chemicals, pollutants and contaminants enter the aquatic environment. Many different toxicants in the environment can suppress or affect the immune system of fish and thus also suppress their protection against disease-causing agents (Reviewed by Anderson, 1995; Wester, 1994; Zelikoff, 1994).

2.4.2 Stress and fish immune system

Stress can be defined as a condition in which the dynamic equilibrium called homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly termed stressors (Chrousos & Gold 1992). Stressful situations result in a cascade of events (a stress response) that are transduced centrally and communicated via the nervous and endocrine systems (Fig 1.). Although the neuroendocrine system responds in patterns characteristic to each stressor, the dominant role of catecholamines and cortisol, the main interrenal steroid in teleosts, in stress response, is generally recognised (Wendelaar Bonga 1997). Unlike mammals, fish have no adrenal cortex and cortisol is produced by cells of the interrenal glands (Chester Jones et al. 1980). In fish, the neuroendocrine circuit involved in the cortisol production is known as the hypothalamus-pituitary-interrenal axis. The release of the stress hormones is known as a primary stress response whereas the secondary responses are usually defined as the manyfold immediate actions and effects of these hormones at the blood and tissue levels. Tertiary responses extend to the level of the organism and population: inhibition of growth and reproduction, immune competence and a reduced capacity to tolerate subsequent or additional stressors.

Through the stress response an animal tries to cope with a stressor by readjusting its biological activities, which implies the reallocation of energy. The action of stressors are twofold: they elicit a coordinated set of behavioural and physiological responses thought to be adaptive, enabling the animal to overcome the threat (eustress) and/or they produce effects that disturb the homeostatic equilibrium (distress). If the animal experiences intense chronic stress, the stress response may lose its adaptive value and become dysfunctional, which may result in inhibition of growth, reproductive failure, and reduced resistance to pathogens.

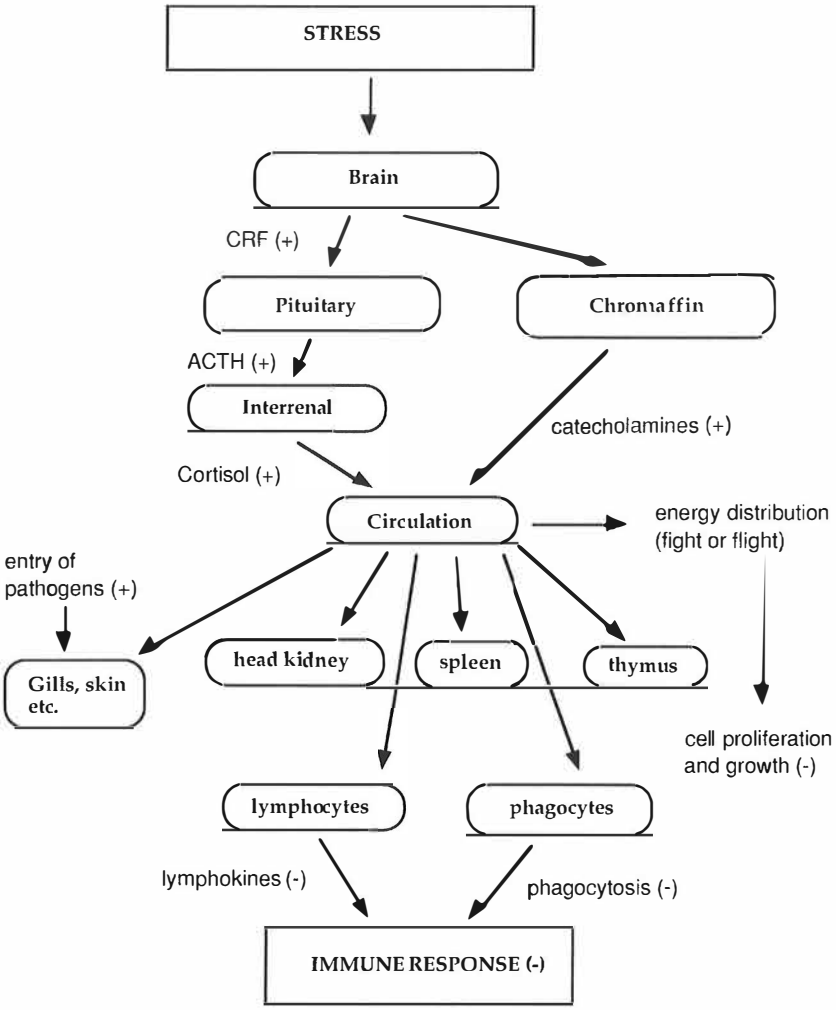

FIGURE 1 Schematic and generalised presentation of the effects of stress on the immune system of fish (source: Schreck, 1996)

The stress-related immunosuppression is in large part mediated via cortisol. The mechanism by which of cortisol acts appears to be through a specific receptor in the leukocytes (Maule & Schreck 1991). Cortisol induces apoptosis in B lymphocytes, but inhibits apoptosis in granulocytes of carp (*Cyprinus carpio*) (Weyts et al. 1998a; Weyts et al. 1998b). It has been thought that cortisol acts as a regulator, inhibiting some parts of the (specific) immune response (Ellsaesser & Clem 1987; Carlson et al. 1993; Espelid et al. 1996) and enhancing other (nonspecific) parts (Peters et al. 1991; Pulsford et al. 1994) that may be functional in stressful situations, and that stimulation of a rapidly occurring nonspecific defence response may be part of an adaptive response necessary to combat potential pathogens under stressful conditions (Weyts et al. 1999). More specifically, in some fish species, some nonspecific defence parameters, such as phagocytosis, have been found to decrease under stress (Narnaware et al. 1994).

In several fish species variety of different stressors (e.g. transport, anoxia, social conflict, handling, injection, crowding) or cortisol treatment results in decreased numbers of circulating B-lymphocytes and increased numbers or circulating granulocytes (reviewed by Wendelaar Bonga, 1997; Weyts et al., 1999).

3 OBJECTIVES

The goal of this thesis was to establish whether the immune system of fish is affected by UVR. A wide array of immunological parameters were investigated in order to gain a comprehensive view of UVR-induced immunological alterations.

The specific aims were:

1) to investigate whether exposure to a single dose of UVB irradiation (I-IV) or UVA irradiation (IV) causes alterations in the immune defence parameters in fish,

2) to study which parts of the fish immune system are most sensitive to the effects of UVB (I-IV) or UVA irradiation (IV), and

3) to investigate how UVR-induced alterations are mediated (II, IV).

4 SUMMARY OF MATERIALS AND METHODS

The materials and methods are described in detail in the original articles (I-IV).

4.1 Fish

Roach (*Rutilus rutilus*) were caught by angling in an oligotrophic lake in central Finland (Lake Peurunka). The protozoan ectoparasites of fish were killed at the beginning of the acclimation period in 300 liter flow-through tanks filled with dechlorinated and aerated tap water at 17-18°C. The fish were fed daily with commercial dry pellets (FinnEwos Aquo Co., Finland) and kept at a 12/12 h light (300-500 lux, no UVR)/dark cycle. Fish were transferred from the maintenance tanks to 120 l (I-III) or 60 l (II-IV) flow-through aquaria at least one week prior to irradiation.

4.2 Experimental UVR exposures

The roach were exposed from above to UVB from two unfiltered Philips TL12 lamps (I-IV) or to UVA from two unfiltered Philips TL05 (IV, for spectral characteristics of the irradiations see fig. 1 in study IV). The ultraviolet radiation penetrating into the water was measured with a UVX Digital Radiometer (Ultraviolet Products Inc., San Gabriel, CA, USA) equipped with interchangeable broadband sensors, UVX-36 (peak sensitivity at 360 nm) and UVX-31 (peak sensitivity at 310 nm). The waterproofed equipment was calibrated with an Optronic 750 spectroradiometer. The dose of UVB received by the free-swimming fish was 0,4-0.5 J/cm^2 given at a mean radiant intensity of 66-100 μW/cm^2 (I-IV) and the dose of UVA was 3,6 J/cm^2 given at a mean radiant intensity of 500 μW/cm^2 (IV).

In study II, a group of roach were exposed to visible light from above delivered with an Osram Dulux S 11W lamp, UVB-depleted with a 6 mm sheet

of clear polyacryl. The illuminance of visible light was measured with a photometer-radiometer HD 9221 (Delta OHM, Padova, Italy) equipped with a photometric probe HD 9221/S1 (Delta OHM). The fish received an illuminance of 1 600 lux for 120 minutes. In the same study (II) another group of roach were subjected to handling stress, which was administered by a 10-second aerial emersion/ 10-second water immersion of netted fish for two minutes. The net-handling protocol was repeated once two hours later.

4.3 Immunisation of fish (III)

In order to study the ability of roach to respond to foreign antigens, the fish were immunised with an intraperitoneal injection of 200 µl containing 500 µg of the immunogen, bovine γ-globulin (BGG, Sigma Chemical Co., St. Louis, USA), in saline and emulsified 1:1 (vol/vol) in Freund's complete adjuvant (Difco Laboratories, Detroit, MI).

4.4 Sampling

The fish were anaesthetised with 0.01% MS-222 (Sigma Chemical CO., St. Louis, USA). A blood sample was collected from the caudal vein using a heparinised 1 ml syringe with a 25-gauge needle. Plasma was stored frozen (-70°C). Cell isolation of the spleen, blood or kidney were carried out using Percoll density gradients after disrupting the spleen and head kidney against a nylon net. The cell culture medium used in all experiments were modified for roach. The viability and the numbers of the cells isolated were counted by trypan blue exclusion in a haemocytometer (viability >95%).

4.5 Immune function assays

4.5.1 Migration (I)

The ability of head kidney granulocytes to move was assayed by a migration-under-agarose technique modified from the method of Nelson et al (1975). The middle wells were filled with casein and the outer wells received neutrophils. Cells were allowed to migrate under the agarose in a humidified environment at 25°C for 3 hr, then fixed overnight with methanol. The agarose was removed and slides with migrated cells were stained. The distance of the leading front of the cells that had migrated from the margin of the well towards the well

containing casein (directed migration) and in the opposite direction (random migration) were measured under the microscope.

4.5.2 Respiratory burst (I-IV)

Whole blood or head kidney phagocytes were stimulated with phorbol 12-myristate 13-acetate (PMA) and the resulting respiratory burst was determined by the luminol-enhanced chemiluminescence (CL) method. CL was monitored with a temperature-controlled luminometer at 25°C. The peak value was taken for the analyses.

4.5.3 Spontaneous cytotoxicity (I, II, IV)

The NCC activity of leukocytes against K562 target cells was determined with a ^{51}chromium release assay. Whole blood leukocytes and isolated head kidney granulocytes were used as effector cells in the assays. Sodium ^{51}chromate (Amersham International plc., Buckinghamshire, U.K.) labelled target cells (K562) were pipetted to round-bottomed 96-well microtiter plates (Nunc Co., Denmark) and effector cells were added. The plates were incubated at 26°C in an atmosphere of 5% CO_2 for 18 h. Supernatants from each well were harvested and counted in a gamma counter (LKB-Wallac RackGamma II 1270, Finland) and percentage cytotoxicity was calculated.

4.5.4 Proliferation assay (III-IV)

Proliferative responses of lymphocytes were measured after mitogen activation (Aaltonen et al. 2000). Lymphocytes isolated from the blood or spleen were added in triplicate to 96-well plates 4 x 10^5 cells /well and activated with Concanavalin A (ConA, 50 µg/ml, Sigma) or lipopolysaccharide (LPS, 150 - 200 µg/ml, Sigma). After 5 days incubation ^{3}H-thymidine was added and the cultures were incubated for 18 hours and harvested by water lysis and adherence to glass fiber filters. The results were recorded as radioactivity counts per minute.

4.5.5 Enumeration of secreting lymphocytes (III)

The enzyme-linked immunospot (ELISPOT) assay was used for enumeration of immunoglobulin-secreting cells (ISC) or antigen-specific antibody-secreting cells (ASC) (Aaltonen et al. 1994). Flat-bottomed 96-well microtiter plates were coated either with BGG, for the determination of specific ASC, or with rabbit anti-roach IgM antibody, for ISC. Cells were dispensed into BSA-saturated wells and allowed to secrete antibodies for 3 hours. Trapped antibodies were detected with biotin-conjugated anti-roach IgM antibody followed by alkaline

phosphatase-conjugated avidin. The substrate, bromo-chloro-indolyl phosphate, was mixed with warm agarose and added to the wells. Finally, blue spots were counted using a stereo microscope.

4.5.6 Quantification of immunoglobulin in blood (III-IV)

The levels of IgM and anti-BGG specific antibodies in the roach plasma samples were determined by enzyme-linked immunosorbent assay (ELISA) (Aaltonen et al. 1994). The assay of serum immunoglobulin was standardised with known concentrations of purified roach IgM, and in the case of the specific antibody, a calibration curve was constructed using a pool of high titer plasmas obtained from fish immunised with several injections of BGG. The concentrations of anti-BGG specific antibodies in the samples were then expressed as artificial units /ml (U/ml).

4.6 Hematological assays (II, IV)

Hematocrits were determined in heparinised 75 mm hematocrit tubes. Blood leukocytes and erythrocytes were counted after staining (Shaw 1930). Briefly, 20 µl fresh blood sample was added to a tube containing 20 µl solution A (0.85 mM Neutral red in 0.15 M NaCl) to which 400 µl solution B (0,294 mM Crystal violet, 0.11 mM sodium citrate and 0.4% formaldehyde) was added. The unstained erythrocytes and blue-stained leukocytes were counted in a hemocytometer. Blood leukocytes were further identified by differential counting. Thin blood smears were prepared from fresh heparinised blood on microscope slides. The smears were air-dried and stained by a modification of the Wright-Giemsa hematological staining procedure (Diff-Quik, Baxter Diagnostic AG, Düdingen, Germany). A total of at least 200 leukocytes were counted and classified as lymphocytes, thrombocytes, granulocytes, monocytes or other, unidentified, cells under a light microscope using a 63x magnification oil immersion objective. The percentage of leukocyte types was calculated.

4.7 Plasma protein and cortisol (II, IV)

Plasma samples were thawed and the protein concentration was measured by the modified Lowry method (Peterson 1977) using albumin as a standard. Plasma cortisol was measured with a commercial radio immuno assay kit.

4.8 Statistics

The data were analysed for statistically significant differences by the Mann Whitney U-test. A statistically significant difference from controls is expressed as $*P \leq 0.05$, $**\ P \leq 0.01$ or $***\ P \leq 0.001$. The tables and figures of the results section represents mean ± standard deviation of the indicated parameter.

5 REVIEW OF THE RESULTS

The data presented in this section are pooled data drawn from studies I-IV and from unpublished experiments with a similar study design. No mortalities or signs of sunburn or infections of the skin were noted during any of the experiments.

5.1 Lymphocyte functions

5.1.1 Proliferation (III, IV)

Polyclonal proliferation of splenic lymphocytes activated with a lectin mitogen ConA or LPS of microbial origin was suppressed following both UVB and UVA irradiation (Table 1). There was very wide individual variation, which is typical of *in vitro* mitogen responses. Proliferation of mitogen-activated blood lymphocytes was suppressed transiently following exposure to either UVB or

TABLE 1 Mitogen-activated proliferation[#] of splenic and blood lymphocytes following exposure of roach to UVB or UVA irradiation

	Control	UVB					UVA		
		Day 1	Day 2	Day 3	Day 7	Day 14	Day 1	Day 7	Day 14
Spleen	(n=65-72)	(n=18-24)	(n=6)	(n=11)	(n=12)	(n=13)	(n=7)	(n=7)	(n=6)
ConA	100 ± 108	68 ± 91	64 ± 47	40 ± 48	47 ± 86*	75 ± 52	26 ± 15	83 ± 108	52 ± 67
LPS	100 ± 104	98 ± 90	52 ± 76	44 ± 33	26 ± 33*	50 ± 51	26 ± 11	29 ± 23	84 ± 45
NIL	100 ± 105	70 ± 49	53 ± 28	40 ± 36	39 ± 32	52 ± 30	95 ± 122	58 ± 49	68 ± 29
Blood	(n=19-24)	(n=6-12)			(n=4)	(n=4)	(n=2-4)	(n=5)	(n=5)
ConA	100 ± 93	82 ± 139			46 ± 52	608 ± 542	27 ± 28	164 ± 205	533 ± 508
LPS	100 ± 91	33 ± 30			53 ± 34	227 ± 328	11 ± 8	51 ± 25	107 ± 123
NIL	100 ± 92	41 ± 27			35 ± 13	165 ± 134	27 ± 6	73 ± 18	110 ± 146

[#] The data is expressed as % of daily control values ± SD.
* $P<0.05$ vs. daily control

TABLE 2 Antibody production# following exposure of roach to UVA or UVB irradiation

	Control	UVB					UVA		
		Day 1	Day 2	Day 3	Day 7	Day 14	Day 1	Day 7	Day 14
	(n=28-94)	(n=14-26)	(n=5-6)	(n=11-12)	(n=7-25)	(n=7-19)	(n=7-14)	(n=7-14)	(n=7-14)
IgM	100 ± 41	107 ± 55	116 ± 58	106 ± 32	91 ± 38	111 ± 36	71 ± 38*	78 ± 33	122 ± 50
ISC spleen	100 ± 37	103 ± 32	172 ± 65*	150 ± 52*	101 ± 54	125 ± 42	76 ± 23	82 ± 23	96 ± 25
ISC blood	100 ± 41	81 ± 39			98 ± 38	66 ± 23	70 ± 29	72 ± 30	84 ± 42

The data is expressed as % of daily control values ± SD.
* $P<0.05$ vs. daily control

UVA (Table 1). However, blood lymphocyte proliferation returned to normal and was even enhanced by the end of the 14-day follow-up.

5.1.2 Antibody production

Total immunoglobulin (III, IV)

UVB exposure slightly increased the plasma concentration of IgM (Table 2). Also, the number of ISC in the spleen increased following UVB exposure, showing a statistically significant increase on days 2 to 3 postirradiation. In contrast, the number of ISC in blood decreased following UVB exposure.

Exposure to UVA decreased IgM levels in roach plasma by 29% on day 1 ($P<0.05$) and by 22% on day 7 postirradiation (Table 2). Also the number of ISC in both the spleen and blood decreased by 18-30% during the first week following to UVA irradiation. However, on day 14 after UVA exposure plasma IgM levels were slightly increased.

Anti-BGG-specific antibodies (III)

The effects of UVB irradiation on the production of antibodies against a novel antigen were assayed by immunising fish with an intraperitoneal injection of BGG emulsified in Freund's complete adjuvant (III). Plasma levels of anti-BGG antibodies increased slightly in UVB-irradiated fish. In addition to plasma antibodies, ASC were enumerated from lymphocytes isolated from the spleen and from the blood. ASC numbers were slightly increased in both lymphocyte compartments following UVB exposure.

5.2 Phagocyte functions

5.2.1 Respiratory burst (I - IV)

Whole blood respiratory burst increased 13-16-fold on days 1 and 2 following exposure to UVB radiation, and remained markedly increased throughout the

14-day follow-up period (Fig. 2). On the other hand, the ability of the head kidney granulocytes and macrophages to produce respiratory burst was markedly suppressed on days 1 and 2 following UVB irradiation. These suppressed functions restored to normal, however, and the respiratory burst by

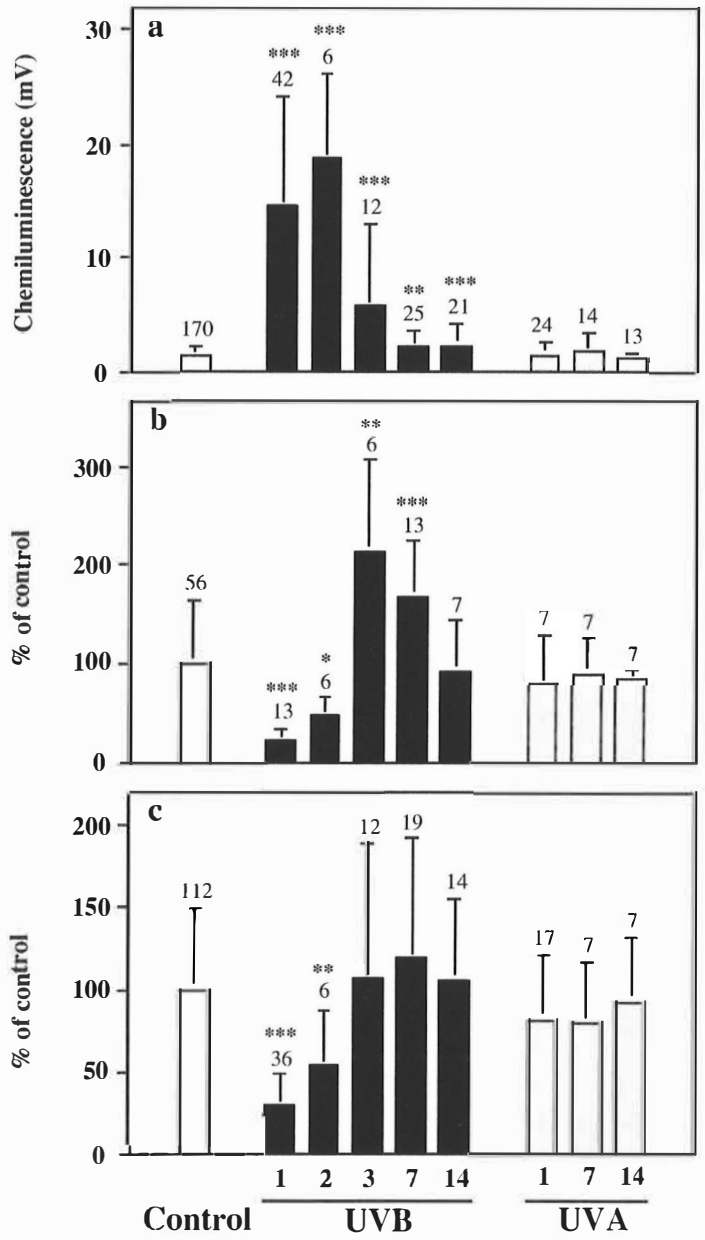

FIGURE 2 Effects of UVB and UVA irradiation on the respiratory burst by a) whole blood, b) macrophages and c) granulocytes isolated from the head kidney of roach. Respiratory burst data for whole blood is expressed as mV, and for head kidney macrophages and granulocytes as % of the daily control value + SD. The numbers of fish are indicated on the top of error bars.

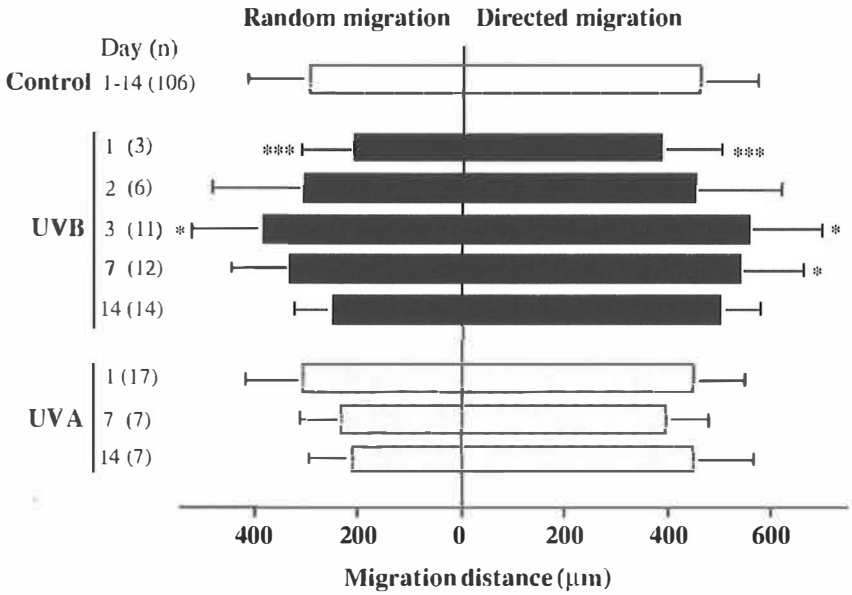

FIGURE 3 Effects of UVB and UVA irradiation on the migration of roach granulocytes. The motility is expressed as migratory distance (mean + SD) in a migration under agarose assay.

macrophages was even enhanced on days 3 and 7 postirradiation. Macrophage respiratory burst returned to the level of controls on day 14 postirradiation.

Exposure of fish to UVA radiation had no effects on the respiratory burst produced by whole blood or by head kidney phagocytes (Fig. 2).

5.2.2 Migration (I)

The ability of the head kidney granulocytes to migrate was suppressed on day 1 following UVB irradiation (Fig. 3). Both random and directed migration were inhibited. The ability to migrate was restored and even enhanced on days 3 and 7 postirradiation.

Exposure of fish to UVA irradiation had no significant effects on the migration of head kidney granulocytes. However, random migration was slightly reduced on days 7 and 14 after UVA exposure (Fig. 3).

5.3 Spontaneous cytotoxicity (I, II, IV)

Spontaneous cytotoxicity by NCCs was assayed in five separate experiments. Table 3 presents pooled data from these experiments. Mean spontaneous cytotoxicity by head kidney granulocytes in control fish varied from 7% to 27% and by blood leukocytes from 13% to 44%. Exposure to UVB decreased cytotoxicity by head kidney granulocytes and blood leukocytes, with one exception:

TABLE 3 Spontaneous cytotoxicity[#] of head kidney granulocytes and blood leukocytes against K562 targets following exposure of roach to UVB or UVA irradiation

	Control	UVB					UVA		
	(n=65-77)	Day 1 (n=26-29)	Day 2 (n=6)	Day 3 (n=11-12)	Day 7 (n=13)	Day 14 (n=14)	Day 1 (n=17)	Day 7 (n=7)	Day 14 (n=6-7)
Head kidney	12 ± 10	8 ± 7	5 ± 4*	6 ± 4*	8 ± 8	7 ± 6	12 ± 8	4 ± 5**	4 ± 4*
Blood	23 ± 19	29 ± 25	6 ± 2*	16 ± 16	18 ± 14	20 ± 11	14 ± 17*	9 ± 11*	13 ± 10

[#] The data is expressed as percent of cytotoxicity (mean ± SD).
* P<0.05 vs. daily control
** P<0.01 vs. daily control

on day 1 postirradiation cytotoxicity by blood leukocytes was increased. Exposure of fish to UVA decreased spontaneous cytotoxicity by both organs, and significantly at two time points out of three during the 14-day follow-up.

5.4 Hematology (II, IV)

Control fish hematocrit 42% ± 7 (n=112), number of red blood cells 1.6 x 10^6 cells/mm^3 ± 0.4 (n=77) and number of white blood cells 46 x 10^3 cells /mm^3 ± 18 (n=77) were not influenced by exposure of fish to UVB or UVA.

Differential counting of blood leukocytes revealed a more than 6-fold increase (P<0.001) in the proportion of granulocytes on day 1 after UVB exposure (Table 4). The proportion of granulocytes remained significantly elevated through the 14-day follow-up, although the increase was not as prominent at the end of the follow-up. On the other hand, the proportion of lymphocytes decreased by 40 % (P<0.001) from control values on day 1 following UVB irradiation. This change was transient and the proportion of lymphocytes did not differ significantly from control values on day 3 postirradiation. UVB exposure had no effects on the proportions of thrombocytes or monocytes.

TABLE 4 Differential leukocyte counts of blood of roach on days 1-14 after exposure to UVB or UVA irradiation

	Control	UVB					UVA		
	(n=139)	Day 1 (n=43)	Day 2 (n=6)	Day 3 (n=12)	Day 7 (n=25)	Day 14 (n=18)	Day 1 (n=23)	Day 7 (n=13)	Day 14 (n=10)
Lymphocytes(%)	60 ± 10	36 ± 15***	38 ± 10***	52 ± 13	56 ± 10	58 ± 9	53 ± 11**	60 ± 8	59 ± 8
Thrombocytes (%)	28 ± 11	28 ± 13	28 ± 9	25 ± 8	28 ± 8	28 ± 7	31 ± 15	29 ± 10	31 ± 8
Granulocytes(%)	5 ± 3	31 ± 14***	27 ± 7***	15 ± 11***	8 ± 5***	6 ± 2*	8 ± 7*	6 ± 3	4 ± 2
Monocytes(%)	4 ± 3	3 ± 3	3 ± 1	5 ± 3	5 ± 5	5 ± 4	3 ± 4	2 ± 1	1 ± 1*
Others (%)	4 ± 3	3 ± 3	4 ± 4	3 ± 2	4 ± 3	4 ± 3	6 ± 5*	3 ± 3	4 ± 3

* P<0.05 vs. control
** P<0.01 vs. control
*** P<0.001 vs. control

UVA exposure also increased the proportion of granulocytes and decreased the proportion of lymphocytes on day 1 postirradiation, but these changes were much weaker than those following UVB exposure and were not observed later in the follow-up. However, UVA increased the proportion of unidentified cells among blood leukocytes on day 1 ($P < 0.05$), and decreased the proportion of monocytes ($P < 0.05$) on day 14 post UVA-irradiation. UVA exposure had no effects on the proportion of thrombocytes.

5.5 Plasma cortisol (II, IV)

The plasma cortisol concentration of all control fish was 552 ± 404 ng/ml (n=111). On day 1 following UVB exposure the mean concentration of cortisol was 195% of the daily control value ($P<0.001$). Thereafter cortisol remained elevated, but the increase was not statistically significant. UVA had no effect on the cortisol level, but on day 1 the standard deviation increased when compared to control fish.

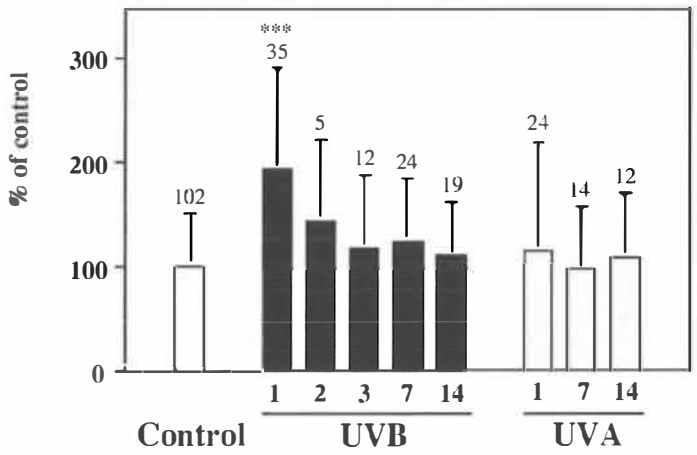

FIGURE 4 Effects of UVB and UVA irradiation on the plasma cortisol level of roach. The daily controls values were assigned as 100% and the cortisol values of UVB- and UVA-exposed fish are compared to them on each day. The numbers of fish are indicated on the top of error bars (SD).

6 DISCUSSION

This research represents the first attempt to characterise the impact of ultraviolet radiation on the immune system of fish. A cyprinid fish, the roach, was the model of this immunotoxicological study. Most of the existing piscine immunological knowledge has been gathered from economically important cultivated species such as salmonids, carp and channel catfish (Wester et al. 1994; Iwama & Nakanishi 1996), but some information also exists about the immune system of roach. The histology of lymphoid organs (Zapata 1981a; Zapata 1981b; Zapata 1982), production of antibodies (Williams & Hoole 1992; Aaltonen et al. 1994; Aaltonen et al. 1997), cellular responses against parasites (Hoole & Arme 1983a; Hoole & Arme 1983b; Taylor & Hoole 1989), phagocytosis (Taylor & Hoole 1995) and mitogen-induced lymphocyte proliferation (Taylor & Hoole 1994) have all been investigated in roach.

Under natural conditions fish can avoid exposure to solar radiation by escaping to deeper waters or into shade. In clear and shallow waters and in fish farming, however, fish are exposed to a considerable amount of solar radiation. The fish in the present study received a dose of 3.6 J/cm^2 UVA or 0.5 J/cm^2 UVB in aquarium during a two-hour irradiation period. The fish sought the dark corners of the aquaria during the delivery of UVR irradiation. This behaviour was taken into account when estimating the doses of UVR the fish had received (I). Similar UVR doses can be received by fish in clear shallow waters during a cloudless summer day (IV). However, comparison between UVR doses produced artificially by lamps and UVR doses in the wild is not straightforward and contains a considerable measure of uncertainty, which is caused by the differences in spectral radiance between lamps and the sun, and in the penetration of UVR into waters with different optical characteristics (Kuhn et al. 1999; Huovinen et al. 2000).

6.1 Proliferation of lymphocytes

Fish possess lymphocytes functionally equivalent, in many respects, to mammalian B and T cells (Iwama & Nakanishi 1996). Proliferative responses to

mitogens are considered as an indicator of the functional status of lymphocytes. In the present study a T cell-specific mitogen, ConA, and a B cell-specific mitogen, LPS, were used to activate roach blood lymphocytes to proliferate *in vitro*. Polyclonal stimulation of lymphocytes by ConA and LPS have been demonstrated also to be selective in fish (Miller & Clem 1988). The lymphoproliferative responses varied greatly between individual roach, as has been observed in other fish species (Tillitt et al. 1988). Both UVB and UVA exposure, irrespective of the type of mitogen used suppressed the proliferation of splenic and blood lymphocytes. During the 14-day follow-up the proliferation of splenic lymphocytes remained suppressed, but the proliferation of blood lymphocytes recovered. Recovery was faster in UVA-exposed than in UVB-exposed fish. It is also noteworthy that LPS-stimulated responses remained depressed over a longer time than the ConA-stimulated responses, suggesting differential effects of UVR on lymphocyte subpopulations in roach.

In vitro exposure of mammalian lymphocytes to UVB has in general resulted in low responses to mitogens and antigens as well as decreased mixed lymphocyte reactions (Spellman et al. 1977; Morison et al. 1979; Lederman et al. 1986; Deeg et al. 1989; Goettsch et al. 1994a). Exposure of rodents and man to UVB, however, have yielded increased (Goettsch et al. 1994a; Guckian et al. 1995), decreased (Morison et al. 1979; Goettsch et al. 1994b) or unchanged (Spellman et al. 1977) lymphoproliferative responses. The reasons for these conflicting results may be connected to the type of radiation source, dosage, recovery time following UVR exposure and species used. Studies on the effects of UVA on lymphoproliferative responses are rare. In mice, a single dose of UVA decreased mitogenic responses (Iwai et al. 1999).

Taken together, the results of the present study imply that exposure either to UVB or to UVA radiation suppresses the proliferation of piscine lymphocytes and suggest that lymphoproliferation recovers more rapidly from UVA-induced suppression than UVB-induced suppression, and that T-like lymphocytes recover from the UVR-induced suppressed stage sooner than B-like lymphocytes.

6.2 Antibody production

The ability of fish to generate an antibody response was assayed by intraperitoneal immunisation of roach with BGG, a thymus-dependent protein antigen (III). Under the conditions used in the present study the anti-BGG-specific ASC response in the spleen of roach peaks on day 21 post immunisation (Aaltonen et al. 1997). Exposure of roach to a single dose of UVB one day prior to immunisation had no marked effect on ASC counts either in the spleen or blood, but an additional three doses of UVB, administered post immunisation, resulted in slightly elevated ASC responses. This was supported by a slightly elevated level of anti-BGG antibody in the plasma (III).

On the other hand, recent studies have demonstrated suppressed production of specific IgG and IgE antibodies in ovalbumin injected rats exposed to UVR (Garssen et al. 1999; van Loveren et al. 2000), suggesting that antibody responses may also be suppressed following UVR exposure, which was not believed to be the case only a decade ago (De Fabo & Noonan 1990).

To study basic immunoglobulin production, IgM levels in plasma and the cells producing immunoglobulins (ISC) were assayed. After UVB exposure, the number of ISCs was increased in the spleen, which is regarded as an important immunoglobulin producing organ in roach (Aaltonen et al. 1994), but decreased in the blood throughout the 14-day follow-up. This trend was evident also on day 22 in BGG-immunised fish (III). Although UVB exposure changed the pattern of immunoglobulin synthesis, stressing the dominance of the spleen, it had no clear net effect on the circulating IgM levels (Table 2, see also study III). In mammalian studies, minor or transient effects of UVB exposure on immunoglobulin production have been found. Morison (1979) noted a slight decrease in the number of circulating surface Ig-positive cells in exposed human subjects, Falkenbach (1997) reported decreased serum IgG but increased IgA concentrations after a vacation to a sunny country, and El-Ghorr (1995) found unchanged serum total immunoglobulin levels in mice during a 6-week low-dose irradiation protocol, but an increase in IgE levels during the first 2 weeks.

UVA irradiation decreased the number of ISCs in both organs investigated in the present study (spleen and blood), but surprisingly, by the end of the follow-up the plasma level of IgM was slightly elevated. A follow-up period of 14 days is possibly too short to assess the ultimate effects of UVA on the production of immunoglobulins, and thus longer term studies are called for. The effects of UVA on fish antibody responses was not assayed in the present study.

The present study suggest that in fish the production of immunoglobulins or specific antibodies does not suffer from exposure to single or repeated doses of UVB over the short term.

6.3 Spontaneous cytotoxicity

The natural cytotoxic cells of fish resemble mammalian natural killer cells (Evans & Jaso-Friedmann 1992). In channel catfish (*Ictalurus punctatus*) the NCCs are small, agranular and lymphocyte-like in appearance and density, but in carp the cells possessing NCC activity are neutrophilic granulocytes (Kurata et al. 1995). Roach granulocytes isolated from the head kidney were spontaneously cytotoxic against K562 cells and can thus be considered as NCCs. However, some cytotoxic activity was also found in cells of buoyant density between 1.050-1.070 mg/mm^3, suggesting that cells other than granulocytes also are capable of NCC activity in roach (data not shown). Roach

blood leukocytes showed cytotoxicity against K562 targets. Whole blood cytotoxicity is a novel parameter in animal studies, where NK/NCC cells are usually isolated from the spleen, or in the case of fish from the head kidney. In human studies, however, the main source of NK cells is the blood.

In man, the majority of studies demonstrate decreased spontaneous cytotoxic activity of blood NK cells after exposure to both UVA and UVB irradiation (Hersey et al. 1983; Hersey et al. 1988; Gilmour et al. 1993; Hersey et al. 1993; Guckian et al. 1995). In rats, splenic NK activity was suppressed after 3 and 7 days of daily UVB doses, but not thereafter during a 42-day follow-up (Goettsch et al. 1994a). Chronic UVB exposure (over 10 weeks) has contradictory effects on splenic NK activity in different strains of nude mice, since both decreased (Toda et al. 1986) and increased (Steerenberg et al. 1997) NK activity has been observed. In the present study, exposure of fish to UVB or UVA decreased the NCCac tivity of the head kidney granulocytes during the succeeding 14 days, and also suppressed the cytotoxicity of the blood leukocytes throughout the 14-day follow-up, except on day 1 after UVB irradiation.

The results of the present study together with those of the mammalian studies indicate that both UVB and UVA radiation can suppress the cytotoxicity of NK or NCC cells in several species, including roach. NK cells are important in the recognition and lysis of both virally infected cells and tumour cells. They also play a part in the development of Th1-like immune responses via the release of IFN-γ. In mammals, NK cells plays an important role in tumour surveillance, and evidence exists that the enhanced ultraviolet-induced impairment of NK function could be partially involved in cancer development (Ishigaki et al. 1998; Miyauchi-Hashimoto et al. 1999). Since fish NCCs participate in protection against viral, parasitic and neoplastic diseases (Evans & Jaso-Friedmann 1992), these defences may be in danger when fish are exposed to UVR.

6.4 Phagocytes

The migration of phagocytes towards an increasing concentration of chemotactic substances often preludes the efficient killing of a pathogen. The other important steps in phagocytosis consist of the adherence of the phagocyte to a microbe, followed by the ingestion and subsequent killing of the microbe. The successful destruction of pathogenic microbes requires the production of reactive oxygen species (ROS) in a process known as respiratory burst. In the present study two parameters related to phagocytosis were assayed: the migration of granulocytes and the capability of phagocytes to produce ROS.

In cyprinid fish the head kidney is the major phagocytic organ (Lamers & Parmentier 1985), and the main organ in producing blood cells (Zapata et al. 1996). In the present study a single exposure of fish to UVB resulted in a

markedly reduced random and directed migration of head kidney granulocytes on day 1 after exposure but thereafter the mobility was similar to that of granulocytes from unexposed fish, or even enhanced (I). ROS production by the head kidney macrophages and granulocytes were also strongly suppressed on days 1 and 2 following UVB radiation (I, IV). ROS production capability by macrophages, but not by granulocytes, was markedly elevated on days 3 to 7 postirradiation (I, IV). At that time, however, the variation in ROS production by the head kidney granulocytes was higher in the irradiated than control fish; e.g. in some fish UVB may continue to have a suppressive and in other fish an enhancing effect on granulocytes. This suggests individual differences in response to UVB radiation among fish. It is not clear why the macrophages and granulocyte pools in the head kidney exhibited such different response patterns to UVB on days 3 to 7. UVA exposure had no effects on the functions of head kidney phagocytes.

In humans, phagocyte migration has been studied using cells isolated from the blood. A single, minimal erythemal dose of UVB resulted in decreased adhesion of phagocytes (Leino et al. 1999), which *in vivo* proceeds the diapedesis of granulocytes from the vasculature to tissues. Human subjects exposed to UVB three times per week for 4 weeks (total dose 43.2 J/cm^2) had lowered blood polymorphonuclear (PMN) leukocyte chemotaxis, but this decrease was no longer detected after 2 weeks' irradiation (Lundin et al. 1990). In contrast, the chemotactic responsiveness of blood PMN leukocytes was enhanced following irradiation with broadband UVR (290 - 400 nm, total dose 20 J/cm^2) five times during 10 days (Csato et al. 1984). Thus the wavelength and the timescale after irradiation may be important in determining the modulation of granulocyte migration in humans as well as in fish.

Many studies have demonstrated that UVB exposure decreases phagocytic activity by phagocytes isolated from inner organs. For example, in mice, exposure to a single UVB dose (1,12 J/cm^2), reduced the phagocytic uptake of bacteria by splenic and peritoneal macrophages on days 3 to 28 after irradiation, but this was no longer evident on day 42 (Jeevan et al. 1995). In the same study, the intracellular killing and ability to produce reactive nitrogen intermediates by peritoneal macrophages were also significantly affected. In both fish and mice, a single exposure to UVB initially suppressed the functioning of phagocytes from the inner organs, after which recovery took place. However, in these mice the suppressed stage of phagocytosis lasted much longer than in the fish studied here. This may be due to the differences in doses and UVB protocols in the two studies as well as in the responsiveness to UVB between these species.

The effects of UVB exposure on phagocytosis by blood leukocytes are in the main suppressive. In rats, for example, the phagocytic activity of blood macrophages was impaired on day 7 after a period of 5 daily UVB exposures (Goettsch et al. 1996b). In man, suppressed phagocyte activities have been demonstrated following UVB exposure (Lundin et al. 1990; Leino et al. 1999), but in experiments where UVB has been the minor and UVA the dominant

component of UVR exposure, enhanced blood phagocyte activity has been reported (Csato et al. 1984; Meffert et al. 1989; Müller et al. 1990; Meffert & Scherf 1992). In the present study, whole blood ROS production increased 13- to 16-fold on the first two days following UVB exposure and remained enhanced throughout the 14-day follow-up. Determination of the activity of individual blood phagocytic cells was not possible because of the difficulty in collecting a sufficient number of erythrocyte-depleted phagocytes, and thus the results obtained by the present method are not directly comparable with those in mammalian studies. However, since no cell isolation is used, this method provides a good estimate of the ability of phagocytosis in the blood of fish in an intact physiological state. The elevated level of ROS production can be explained by the simultaneous increase in the proportion of granulocytes in the blood. The increase in granulocytes was 6-fold on day 1 and was also markedly elevated at the end of the follow-up.

These results demonstrates that in fish UVB radiation modulates the migration and phagocytosis of granulocytes and macrophages isolated from the head kidney. The present study also indicates an increase in the potential to destroy invaders in the blood of UVB-irradiated fish. The way the UVR exposure is delivered (wavelength, single/multiple exposures, high/low doses), recovery time after exposure(s) and species may be important in determining whether the outcome will be the suppression or enhancement of phagocyte function parameters. In rodents, exposure to UVB during infection can lead to an increase in the number of microbes in the inner organs due to diminished phagocytosis and phagocyte killing capacity thus leading to exacerbation of the disease (Jeevan et al. 1995; Goettsch et al. 1996b). It seems possible, that also in fish infections may be exacerbated in a similar manner after UVB exposure.

6.5 Hematology

A single exposure to UVB had no effects on the hematocrit or on the number of red or white blood cells in roach. In study IV, UVA slightly decreased these parameters on day 1 postirradiation, but when the hematological results from all the studies where fish were exposed to UVA were pooled (see chapter 5.4) no changes in the hematocrit or in the number of red or white blood cells were observed during the follow-up period. Chronic UVB exposure, on the other hand, induced a small increase in the total number of white blood cells of chickens (Kosar 1974) and a significant increase in mice (Flindt-Hansen & Ebbesen 1991) , but did not alter the total number of white blood cells in man (Meffert et al. 1989) or in opossums (*Monodelphis domestica*) (Kusewitt et al. 1996).

In the differential counting, blood leukocytes were identified on the basis of their morphological and staining properties (Houston 1990; Fänge 1992).

Although UVB did not change the total number of fish blood leukocytes, the proportion of granulocytes increased and the proportion of lymphocytes decreased very markedly. Exposure to UVA also increased the proportion of granulocytes and decreased the proportion of lymphocytes, although the changes were much lower in magnitude when compared to those induced by UVB. Spangrude (1983) found that UVR exposure of mice resulted in a dramatic and long-lasting increase in the tropism of peripheral lymph nodes for circulating lymphoid cells and concluded that the enhanced transport of lymphocytes into the peripheral lymph nodes of UVR-exposed mice occurs primarily via lymphocyte-high endothele venule interactions. In the present study, the ISC results indicate the transport of secreting lymphocytes from blood to spleen following both UVB and UVA exposure. Thus certain kind of similarity exists in the distribution of lymphocytes in mice and roach following UVR exposure.

Hematological changes such as leukopenia, lymphopenia, and neutrophilia in fish may be the results of stress and are linked with increased cortisol values (Ellsaesser & Clem 1986; Bly et al. 1990; Ainsworth et al. 1991). All of the above-mentioned hematological changes and increase in plasma cortisol were also observed in the handling-stressed roach (II). Furthermore, all these alterations, except blood leukopenia, were even more prominent in the UVB-exposed roach. Thus the alterations in the proportions of blood leukocytes and increased cortisol values in UVB-exposed fish may have resulted, at least partly, from a UVB-induced stress response. Exposure of fish to UVA induced some stress-related hematological changes but did not, for instance, increase the plasma cortisol level.

In mammals, increased granulocyte counts have been demonstrated in mice (Gahring et al. 1984) and rabbits (Spode 1974) after a single UVB dose and in man (Lundin et al. 1990), mice (Ebbesen 1981; Flindt-Hansen & Ebbesen 1991) and chickens (Kosar 1974) after chronic exposure to UVB. Decreased lymphocyte counts have been detected after chronic UVB exposure in man (Hersey et al. 1983), rats (Goettsch et al. 1994a) and chickens (Kosar 1974). Thus the changes in the blood leukocyte composition found in present study are in line with those found in mammalian studies.

In addition to the changes in lymphocytes and granulocytes, UVA exposure also increased the proportion of cells that fell in-between lymphocytes and monocytes in the differential counting and were classified as unidentified cells. The distinction between lymphocytes and monocytes is difficult in some other fish species as well (Houston 1990). UVA penetrates deeper into the mammalian skin than UVB, and this most probably also holds for fish skin. Lymphocytes are very sensitive to the damaging effects of UVR (Arlett et al. 1993; Tomimori et al. 2000) and it is possible that the unidentified cells may be lymphocytes damaged by exposure to deeply penetrating UVA.

6.6 Mechanisms in UVR-induced immunomodulation in fish

Although an extensive body of studies have investigated the mechanisms of UVR-induced immunomodulation in mammals, the precise mechanisms are not known at present. However, several pathways leading to immunosuppression have been depicted in mammals. The mechanisms of immunomodulation have not been investigated in fish.

DNA and UCA are considered to be the primary chromophores in mammalian skin (Noonan & De Fabo 1992; Vink et al. 1996). In the skin of platyfish (*Xiphophorus*) UVB radiation induces DNA damage more efficiently than UVA radiation (Ahmed & Setlow 1993), implying a role for DNA as a UVR chromophore also in fish skin. It has been demonstrated that both UVB and UVA radiation are capable of inducing the *trans* to *cis* isomerisation of UCA in human skin and in aqueous solution *in vitro* (Kammeyer et al. 1995). However, only *trans*- but not *cis*-UCA was detected in the dorsal skin of UVB-exposed rainbow trout (*Oncorhynchus mykiss*) (Fabacher et al. 1994).

In the process in which the energy of a photon in the UVR range is absorbed, reactive oxygen radicals are formed, and these can cause oxidative stress to the molecules of a cell (Tyrrell 1991; Kohen et al. 1995). It has been postulated that oxygen radical generation is the major mechanism by which UVA damages the biological system (Halliday et al. 1998). Since antioxidants can inhibit the induction of UVR-induced immunosuppression, such as CHS and antigen presentation, it is believed that oxidative stress may be one mediator of UVR-induced immunosuppression in mammals (Katiyar et al. 1995; Clement-Lacroix et al. 1996; Iwai et al. 1999). UVB exposure reduces skin and muscle glutathione concentrations in zebrafish (*Brachydanio rerio*), indicating that UVB-induced oxidative stress also occurs in fish species (Charron et al. 2000).

Cytokines mediate UVB- and UVA-induced immunomodulation in mammals (Krutmann 1995; Luger & Schwarz 1995). For example, UVB induces the release of histamine from dermal mast cells in mammals (Hart et al. 2000). Histamine and cis-UCA induce the production of eicosanoids, for example prostaglandin E2 (PGE2) (Jaksic et al. 1995), which among other immunosuppressive effects (Phipps et al. 1991) may in turn trigger a cytokine cascade (PGE2 --> IL-4 --> IL-10) that ultimately results in systemic immune suppression (Shreedhar et al. 1998). EGCs are mast cell equivalents in the fish skin, and are also able to secrete histamine (Vallejo & Ellis 1989). Eicosanoids are also well known in fish (Secombes 1996), and thus fish skin possesses several compounds similar to the mammalian mast cell-mediated cascade leading to immunosuppression. Although cytokines and cytokine secreting cells are still largely unknown in fish (Secombes et al. 1996), cytokines such as IL-1 and transformic growth factor β have been cloned (Secombes et al. 1998; Secombes et al. 1999), and cross-reactivity to mammalian TNF-α and IL-6 has

been reported, suggesting that these cytokines are also present in fish (Secombes 1996).

The UVB-induced immunomodulation found in the present study is well in accordance with the known impact of stress on the immune parameters in fish. For example, suppressed proliferative responses of lymphocytes to mitogenic stimuli, also observed in the present study, have been documented in fish stressed by e.g. handling (Ellsaesser & Clem 1986) or by social confrontation with aggressive fish (Faisal et al. 1989). Decreased NCC activity has resulted from social confrontation stress in tilapia (Faisal et al. 1989), and is seen in the present study. The effects of stress, or cortisol, on the phagocytes of fish are contradictory since both suppressed and enhanced effects have been found (reviewed by Weyts, 1999). The present study demonstrates suppressed activity by head kidney phagocytes following exposure to UVB.

UVA exposure also suppressed lymphocyte proliferation and NCC activity and induced stress-related cellular changes of the blood, i.e. increased number of granulocytes and decreased number of lymphocytes, but cortisol values were not increased. However, since in fish a stress-induced increase in cortisol levels may return to basement level by even 4 hours following the stress (Pickering et al. 1982) and in the present study the first sampling point was on day 1 (24 h) postirradiation, it could not be entirely overruled that also UVA, although in much lesser amplitude than UVB, may bring about a stress response in fish. On the other hand, in mammals and most probably also in fish, tissue transmission increases with wavelength allowing UVA to reach targets well below the surface of the skin, and it is possible that a small quantity of long wavelength UVR is absorbed by blood components (Tyrrell 1996). Differences in the ability to penetrate into the skin may be responsible for the different effects following exposure to UVA and UVB irradiation.

Sensory perception of the stressor is a prerequisite for eliciting a stress response, in fish as well as in other vertebrates (Wendelaar Bonga 1997). Roach can see in the UVA region (Douglas 1986) and most probably sensed the radiation from both UVA and UVB broad-band lamps used in the present study via the eyes. However, the amount of visible light was not sufficient to induce immunological changes in roach (II), suggesting that the skin plays a pivotal role in fish, as in mammals, in UVR-induced immunosuppression.

Only a few studies have discussed stress as a mechanism in UVR-induced immunosuppression in mammals (Slominski & Mihm 1996). In mammals, IL-1 and IL-6, which are UVR-inducible cytokines, can act on the HPA-axis to generate adrenocorticotropic hormone (ACTH) and, subsequently, induce the production of cortisol (Besedovsky et al. 1986; Naitoh et al. 1988). Other mediators related to the HPA-axis, such as catecholamines and neuropeptides, might also have a role in the systemic modulation of the immune system after exposure to UVB (Benrath et al. 1995; Luger & Schwarz 1995; Garssen et al. 1998b; Luger 1998). Interestingly, it has been proposed that an equivalent to the HPA-axis operates in mammalian skin as the main coordinator and executor of the peripheral response to stress (Slominski & Mihm 1996). In response to UVB

skin cells produce POMC and POMC derivatives such as melanocyte stimulating hormones (MSH) and ACTH. These hormones play a key role in the regulation of pigmentation (Slominski & Pawelek 1998) in many species, including fish (Suzuki et al. 1997; Hoglund et al. 2000), and they also modulate secretion of cytokines and αMSH, which is a potent immunosuppressor (Lipton & Catania 1997; Luger et al. 1998). The skin of some fish species is capable of producing POMC and POMC-peptides. This has been demonstrated for example in African lungfish (*Protopterus annectens*) (Masini et al. 1999). However, there are no studies of the induction of POMC or POMC-peptides in the skin of fish by UVR or their effects on fish immune cells.

6.7 Significance of the findings

Taken together, it is obvious that both UVA and UVB radiation have notable effects on the immune system of fish. Immunological and hematological changes together with increased plasma cortisol levels support the consideration of UVB as a potential environmental stressor. The immune response in fish can be impaired by a wide range of environmental factors (Zeeman & Brinley 1981; Anderson & Zeeman 1995) and once the immune system has been suppressed, fish may become more susceptible to infections by pathogens (Anderson 1990). The suppression of fish immune defence by exposure to UVB or UVA, as demonstrated in this study, did not lead to infections during the two-weeks follow-up. However, prolonged exposure of cage-reared Atlantic salmon (*Salmo salar*) to solar UVR led to *Vibrio* spp. and mycobacteria infections in skin lesions (McArdle & Bullock 1987). In addition, fungal pathogens (*Saprolegnia* sp.) have been isolated from the dorsal fins of UVB irradiated rainbow trout (Fabacher et al. 1994). In mammals impaired resistance of the host to infectious agents is related to impaired immune defence following exposure to UVB (Jeevan et al. 1995; Goettsch et al. 1996a; Goettsch et al. 1996b). These findings together suggest that this may also be the case in fish species.

However, it is not clear whether UVR-induced alterations of immune parameters can lead to compromised disease resistance in fish, and further research is needed to elucidate this aspect. Long term studies are needed to elucidate whether fish are able to accommodate to stress and other injurious effects caused by increased levels of UVB, or whether the changes remain permanent or are even exacerbated, leading to compromised immune status.

7 CONCLUSIONS

The focus of this research was to assess the effects of a short-term exposure to ultraviolet radiation on the immune defence of fish. The main conclusions are the following:

1. Proliferation of T- and B-lymphocytes was suppressed after exposure to both UVB and UVA.

2. Antibody-mediated immunity was not affected.

3. NCC activity by fish leukocytes was reduced following both UVB and UVA exposure, but phagocyte functions were suppressed following UVB exposure only.

4. Major changes in the cellular composition of blood leukocytes was observed. UVB induced granulocytosis and lymphocytopenia in blood.

5. UVB brings about a strong stress response, which may mediate UVB-induced immunomodulation in roach.

6. The results indicate that UVB and also, to a lesser extent, UVA radiation is potentially deleterious to the immune system and consequently to the disease resistance of fish.

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YHTEENVETO (Résumé in Finnish)

Ultraviolettisäteilyn vaikutus kalan immunologiseen puolustusjärjestelmään

Yläilmakehän otsonikato on johtanut ultraviolettisäteilyn lisääntymiseen maanpinnalla. Otsonin väheneminen lisää etenkin ultravioletti B (UVB) säteilyn ja vähäisessä määrin myös ultravioletti A (UVA) säteilyn määrää. UV-säteilystä on hyötyä mm. D-vitamiinin muodostuksessa sekä joillakin eläimillä, jotka näkevät UV-valoa. Ihmisen kannalta UV-säteilyn haitoista kenties tunnetuin on ihosyöpien lisääntyminen. UV-säteilylle altistuminen johtaa nisäkkäillä immuunipuolustuksen heikentymiseen, jonka on osoitettu olevan osatekijänä ihosyöpien muodostumisessa ja erilaisten mikrobitautien taudinkuvan pahenemisessa. Koska UV-säteily tunkeutuu veteen, sen vaikutukset ulottuvat myös vesiekosysteemeihin.

Tämä tutkimus osoittaa, että ultraviolettisäteilylle altistuminen voi aiheuttaa muutoksia myös kalojen immunologisen puolustusjärjestelmän toimintaan. UVB-säteilylle altistetuilla särjillä proliferaatiotestillä mitattu lymfosyyttivälitteisen puolustushaaran toiminta heikentyi hieman, tosin kyky tuottaa vasta-aineita pysyi säteilyttämättömien verrokkikalojen tasolla. Tämän lisäksi loisten, virusten ja syntymässä olevien kasvainten vastaisessa puolustuksessa tärkeiden luonnollisten sytotoksisten solujen aktiivisuus heikkeni. Bakteereita tuhoavien fagosytoivien solujen, granulosyyttien ja makrofagien, toimintakyky heikkeni voimakkaasti UVB-altistuksen jälkeen. Särjen veren valko- ja punasolujen kokonaismäärät pysyivät muuttumattomina, mutta eri valkosolutyyppien suhteelliset osuudet muuttuivat siten, että granulosyyttien osuus lisääntyi huomattavasti, mutta lymfosyyttien osuus väheni. UVA-säteily ei kokeissa käytetyllä annoksella annoksella vaikuttanut fagosyyttien toimintaan, mutta lymfosyyttien ja luonnollisten sytotoksisten solujen välittämä puolustus heikkeni. Näinollen altistuminen UVB- tai UVA-säteilylle heikentää kalojen immuunipuolustuksen eri parametrejä.

UVB-säteilyn aiheuttamat muutokset särjen immunologisessa puolustuskyvyssä näyttäisivät välittyvän ainakin osin stressireaktion kautta.

Tätä ajatusta tukevat UVB:lle altistuneiden särkien kohonnut plasman kortisolipitoisuus sekä edellä mainitut valkosolujen erittelylaskennassa havaitut muutokset.

Immuunipuolustus koostuu kalojenkin tapauksessa päällekkäisistä puolustusmekanismeista, jotka osittain voivat korvata toisiaan. Näinollen yhden puolustusmekanismin heikkeneminen ei välttämättä johda heikentyneeseen taudinvastustuskykyyn. Kuitenkin useamman puolustustekijän heikkenemistä, kuten tässä tutkimuksessa havaittiin, voidaan pitää uhkana kalan terveydelle. On vielä huomattava, että auringon ultravioletti-säteilyn spektri eroaa tässä kokeessa käytetyistä lamppujen spektristä. Lisäksi luonnossa kaloilla on myös useimmiten mahdollisuus paeta ultraviolettisäteilyä varjoon tai syvempiin vesiin. Näin ollen tämän tutkimuksen tuloksista ei voida vetää suoria johtopäätöksiä luonnontilaisia kaloja koskeviksi. On kuitenkin selvää, että ultraviolettisäteilyä on pidettävä potentiaalisesti vaarallisena myös kalojen terveydelle, varsinkin lajeilla, jotka eivät ole sopeutuneet korkeisiin säteilytasoihin. Näihin kuuluvat etenkin napaseuduilla tai niiden läheisyydessä elävät lajit, sillä ultraviolettisäteilyn lisääntyminen on voimakkainta juuri näillä alueilla.

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ORIGINAL PAPERS

I

Ultraviolet B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae) I: Phagocytes

Harri Salo, Tuula Aaltonen, Eveliina Markkula & Ilmari Jokinen

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II

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IV

Comparative effects of UVA and UVB irradiation on the immune system of fish

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