Chapter 1

Introduction
Estrogen is a female sex hormone with profound effects on the reproductive system, central nervous system (1), skeletal system (2), cardiovascular system (3) and the immune system (4). It is a known immunomodulator (4) with both pro- and anti-inflammatory effects on various cell types of the immune system. It is believed to be one of the determinants of sexual dimorphism in the immune responses observed between males and females, for example, a gender bias has been observed in the occurrence of autoimmune diseases with a higher incidence of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), autoimmune thyroiditis, etc. observed in women (5). Also, disorders such as multiple sclerosis & rheumatoid arthritis are ameliorated during pregnancy and estradiol treatment, while SLE is aggravated. In general, it has been observed that humoral mediated autoimmune diseases are aggravated during pregnancy while cell-mediated autoimmune diseases are ameliorated suggesting hormonal modulation of systemic immunity. Sexual dimorphism has also been observed in the incidence and progression of various infectious diseases such as listeriosis (6, 7), toxoplasmosis (8), leishmaniasis (8), pulmonary infections, sepsis (9) etc. indicating the possible role of sex hormones in immune alteration.

Macrophages, the cells of the mononuclear phagocyte system, derived by the differentiation of monocytes play a central role in innate and adaptive immune responses and their activation is central to the outcome of virtually every infectious disease. Failure to properly regulate macrophage function during infection is often itself a major cause of disease. These cells play a major role in diverse processes such as antimicrobial activity, wound healing & repair, scavenging of dead cells (10), morphogenesis and organ development (11), tumor cell lysis, tumor initiation & progression (12), antigen presentation, and modulation of T- & B-lymphocyte activity (13), thus serving as vital adapters between the innate and adaptive immune system. The modulation of macrophage survival and function by estrogen has been linked to the pleiotropic effect of this hormone on the immune system.

Macrophages express estrogen receptors and are therefore capable of responding to increase in estrogens during follicular phase of menstrual cycle (14), at the time of exposure from exogenous sources such as phytoestrogens (15), following administration for prophylactic and therapeutic purposes (16) or during accidental
exposure to estrogenic chemicals (17,18). Estrogen affects a variety of macrophage functions, for example it can reduce accumulation of cholesteryl esters in macrophages (2), stimulate production of nitric oxide (19, 20), increase arachidonic acid release (21), regulate activation related events (2, 22), decrease monocyte adhesion to vasculature (23), enhance macrophage phagocytosis (24) and facilitate Ca\(^{2+}\) influx (20) some of which are implicated in mediating the gender bias observed in numerous autoimmune (25), cardiovascular (26) and neurodegenerative disorders (27). In addition, estrogen is able to modulate macrophage death and this event is of great relevance because macrophage survival or death is crucial for disease pathogenesis, however, data available on the influence of the hormone on macrophage cell death process is in itself contradictory. Existing literature show that macrophage-like U937 cells undergo apoptosis when exposed to estrogen (28) but the same cell type is protected from TNF-alpha-induced apoptosis by the hormone (29). According to other reports, estrogen is able to induce apoptosis in undifferentiated U-937 monocytes but macrophages differentiated from these cells are refractory to such effects of estrogen (30). Further examples of cell types where estrogen is reported to induce cell death include bone macrophages like murine osteoclasts, preosteoclastic FLG 29.1 cell line and mouse peritoneal macrophages (2, 31-33).

The apparently paradoxical effect of estrogen on apoptosis in cells of the monocytic lineage could be interpreted to be the result of its ability to differentially modulate anti and pro-apoptotic proteins like the members of the Bcl-2 family that share sequence-homology domains within the group. The various pro and anti-apoptotic Bcl-2 family members are able to heterodimerize and their relative concentrations function as a rheostat for the apoptotic program (34). Certain apoptotic stimuli like exposure to nitric oxide (35), oxysterol (36) and activation inducing agents increase macrophage Bcl-2 or other members of Bcl-2 family of proteins like Bfl-1 (37), but the involvement of Bcl-2 family members in regulating the macrophage death pathway is not understood. In the context of tumor development, mechanisms regulating macrophage death are important because these cells constitute a large proportion of the tumor cells and are evidently important for either progression or regression of tumors (12). Survival of macrophages in estrogen microenvironment is relevant especially in the cells populating estrogen target tissues like uteri, breast, brain and cervix. Also, an understanding of the mechanism of macrophage survival
under altered Bcl-2 level becomes important in the backdrop of development of Bcl-2 small molecule inhibitors, antisense oligonucleotides and RNAi against Bcl-2 intended to be used for treatment of resistant carcinoma and some of which are currently in phase I and phase II clinical trials (38-40). In addition, estrogens and the various selective estrogen receptor modulators (SERM) are in various phases of clinical trial or are already in use for prophylaxis or treatment of various disorders like cancers, osteoporosis, stroke, atherosclerosis, multiple sclerosis, Alzheimer's disease etc., and hence a better understanding of the effect of this hormone on the immune cells is imperative.

Also, the contradictions in the literature regarding the effect of estrogen on macrophage survival is largely due to the fact that the data has been collected from studies performed on mouse or rat macrophages and the various human monocyte-macrophage cell lines overlooking the fact that the effect of estradiol is highly cell type specific and dose dependent and thus cannot be directly extrapolated to human macrophages. While human macrophage cell lines should be used as model systems for investigations because they are amenable to genetic manipulations and available in larger numbers, it is imperative to make comparisons with ex-vivo human peripheral blood monocytes and monocyte-derived macrophages at every step.

Based on the existing literature on estrogen effect on macrophage survival that demonstrate wide gaps in understanding of the influence of estrogen on death pathways in macrophages, the overall theme of the thesis is proposed to understand the role of estrogen in macrophage biology under physiological as well as pathological conditions like infection. In addition, a dissection of the signaling mechanisms involved in estradiol regulation of macrophage function and identification of key players that are vital for modulating human macrophage survival under estrogen exposure would be carried out. Though estradiol is predominantly a female hormone, it does not necessarily restrict the scope of this study to female physiology due to the widespread exposure of mankind to phytoestrogens as well as the fact that there is continuous local biosynthesis of estradiol by the macrophage due to their aromatase activity (41).
The specific objectives of the proposed study are:

1. Investigate the effect of estrogen on human monocyte-macrophage survival and define estrogen receptor involvement in the process.

2. Identify the members of the Bcl-2 family of proteins involved in macrophage response to estrogen and explore their role in modulation of macrophage survival and function.

3. Elucidate the signaling pathways operative under estrogen exposure leading to modulation of the identified Bcl-2 family members.

4. Determine the effects of estrogen on macrophage response to leishmanial infection.

References


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