THE ROLE OF CYTOKINES IN LANGERHANS CELL MIGRATION FOLLOWING TREATMENT OF MURINE SKIN WITH ANTI-CD80 ANTIBODY

By

PRATIMA DESHPANDE

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirement for
the Degree of
MASTER OF SCIENCE
December, 2001

THE ROLE OF CYTOKINES IN LANGERHANS CELL MIGRATION FOLLOWING TREATMENT OF MURINE SKIN WITH ANTI-CD80 ANTIBODY

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. Kim Burnham for patiently and cheerfully addressing all my "dumb" questions. I deeply appreciate his guidance and his positive attitude. I owe him my career choice. I would like to extend my thanks to the members of my committee- Dr. Jeff Hadwiger and Dr. Alexander Lai for their advice and helpful suggestions. I am also grateful to them for allowing me the usage of their lab equipment.

Special thanks to all the members of the staff, the faculty and friends in the Department of Microbiology, for their encouragement and their goodwill. My appreciation to my friends at OSU and to all my past and current roommates for tolerating my mood swings influenced by the frustrations in the research work. Thank you guys for being there for me. I am indebted to my siblings Rajan and Yogita for their affection and their continual support, and to all the members of my extended family for their best wishes.

Finally, I would like to dedicate this work to my parents. Thanks amma and baba, this work would not have been possible without you.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Immunobiology of the Skin

Skin is a highly organized dynamic system where cells constantly divide, selectively migrate, differentiate and die. All these activities make skin a sturdy physiological barrier to harsh environments. Although the skin acts primarily as a dry mechanical barrier by constantly ridding itself of contaminating organisms by means of desquamation, it also adapts itself to various roles with great versatility. It aids in thermo-regulation, acts as a sense organ, is a source of endocrine mediators, and initiates and sustains various immune responses.

Until the late 1960s, skin was not recognized as an organ capable of immune responses. In the 1970s, Streilein noted that skin has afferent and efferent lymphatics, lymphocytes, WBCs and dendritic cells and is hence capable of immune functions (114). Later Margaret Kripke and her coworkers showed for the first time that UV light causes tumors in the skin of mice by local immunosuppression (105). Fichtelius described skin as a primary-lymphoid organ in 1970 (63a), but his concept remains unproved. Though no specific evidence exists for categorizing skin as either a primary or a secondary lymphoid organ, its involvement in the generation and the regulation of immune reactions has led to the coinage of various terms to describe a relationship between skin and immunology. The foremost term that was used in the literature to refer to this relationship was Skin Associated Lymphoid Tissue (SALT) introduced by Streilein and his co-workers in 1978 (202). In 1986, Bos and others proposed the term Skin Immune System

(SIS) to emphasize the role of skin in immunology (25). In 1989, Sontheimer used the term Dermal Microvascular Unit (DMU) to focus on the center of immunological activity (25). Nickoloff in 1993, proposed Dermal Immune System (DIS), a concept that relates to the cellular and humoral aspects of SALT (25). Whatever the term used, the main point is that skin actively participates in immune surveillance. The concept of SALT encompasses or accounts for the presence of cells capable of ingesting, processing and presenting antigens, the presence of specific peripheral lymph nodes for reception of immune-related stimuli, the existence of T cells capable of directional movement and production of immuno-regulatory molecules such as cytokines and chemokines (202). The constituents of SALT are keratinocytes (KC), langerhans cells (LC), epidermal T cells, vascular endothelial cells, lymphatic endothelial cells and draining lymph nodes. To describe the complexity of immune response associated cells, SIS system is more valid. The cellular components of SIS are LC, KC, macrophages, granulocytes, mast cells, endothelial cells and T cells while their humoral components are anti-microbial peptides, complement proteins, immunoglobulins, cytokines, coagulants, prostaglandins and free radicals (24). Thus, it includes both the innate and adaptive parts of immunity.

Some of the facilitators of a successful immune response in skin include antigen presenting cells (APCs), accessory cells, immunocompetent lymphocytes and soluble mediators. KCs comprise the major portion of the all epidermal cells (approximately 90 to 95%) and possess the ability to elaborate a number of cytokines that modulate immunological reactions (128, 110, 75, 102). Cytokine production by KCs reflect on their possible role as accessory cells as well as the fact that KCs can be induced to express Ia antigens, intercellular adhesion molecule 1 (ICAM-1) and other surface molecules under certain diseased conditions such as graft versus host disease (GVHD), allergic contact

dermatitis, lichen planus and inflammatory dermatoses (10) or in response to certain pro-inflammatory cytokines (54). In short, KCs have all the relevant signals needed for accessory cell function but whether they function as accessory cells remains to be seen. As opposed to KCs, LCs comprise only a minor portion of epidermal cells though their role as inducers of immune reactions is indisputable. LCs belong to a class of professional APCs that have a unique trait of inducing primary immune responses (201). They are strategically located in the epidermis, a region that is continuously exposed to innumerable antigens, posing as sentinel cells. They pick up antigens, process them and put them up on the cell surface prior to journeying down to the afferent lymphatics via endothelial cells. The intermittent cohesion and separation of various adhesion molecules expressed on both immune-related cells and endothelial cells facilitate transendothelial cell trafficking. Endothelial cells not only aid in LC migration but also in lymphocyte homing to skin and other organs (154). The occurrence of the synchronized spatial and temporal events necessitates interaction with endothelial cells in a very complex manner. In addition to these cells participating in cutaneous immunity there are dendritic epidermal T cells in murine epidermis that may play a role in induction of tolerance and immune surveillance (226), and dermal dendritic cells, mast cells and macrophages, which may provide an interface with the external environment. All these cells and the interactions among them, by the way of soluble mediators and surface molecules, give the complete picture of immunophysiology of skin.

Layers of the Skin

Skin is one of the largest, one of the most structurally diverse and the most visible organ of the body. It is in direct contact with the external environment, which governs its

functions and determines its structure. It can be defined as a multifunctional system that sheaths and protects the body both passively as well actively. Skin or integument can conveniently be classified into four zones, the ectoderm-derived epidermis, the mesoderm-derived dermis, separated from each other by a junction, basement membrane and subcutaneous fatty tissue. It also includes glands and its various modifications. The outermost layer of the skin that is always in a state of constant renewal is epidermis. It is multi-layered tissue, externally composed of cornified squamosal epithelium. The five distinct layers of epidermis working downwards are stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale, also known as stratum germinativum. Stratum corneum is usually composed of flattened, squamous, hexagonal keratinized dead cells arranged in peculiar columns (37). Cell proliferation and replacement are hormonally regulated (232). It essentially prevents desiccation, modulates temperature changes and protects from exogenous agents. The next layer, stratum lucidum, has compactly packed cells with degenerate nuclei and indistinct cell borders. By the time the cells reach up from germinal layer, they lose their full set of essential organelles. Stratum granulosum consists of four to five layers of polygonal cells with rounded nuclei and endoplasmic keratohyalin inclusions, which were speculated to be precursors of keratin. The layer below the granular layer is the stratum spinosum with its polyhedral shaped cells. These cells when separated exhibit "spiny" morphology hence the name. The cells in this layer are in contact with each other via intercellular bridges, desmosomes and tonofibrils. They show active division but less frequently than the cells in the layer beneath it, the basal layer. The keratinocytes as they pass this layer undergo a process called keratinization.

Stratum basale is the chief cell-producing layer of the epidermis. The cells in this layer appropriately have more mitochondria and high enzymatic activity. The high level of growth and division is supported by an easy access to nutrients from neighboring dermal blood vessels. The cells are columnar or cuboidal in shape with their one surface resting on the basement membrane. Keratinocytes grow from basale and acquire connective junctions as they move up, which helps them keep anchored to each other. LCs reside in the basal and the supra-basal region attached to the neighbouring KCs via E-cadherin and Ca^{++} dependent mechanisms.

The dermis is directly present beneath the epidermis and is separated from it by a non-cellular basement membrane, which serves to prevent the movement of materials and cells across the junction (200). Dermis consists of connective tissue, collagen, reticulin, elastin fibers, fibrocytes, tissue macrophages, mast cells, nerves, lymphatics and blood vessels. Dermis has two indistinct layers: stratum papillaris, the papillary layer and stratum reticularis, the reticular layer. The former layer is far richer in cells, blood vessels, nerves and extra-vascular leukocytes than the latter. In most inflammatory conditions, the responses are always greater in papillary layer than in reticular layer. The dermal layer is essential for the viability of epidermis, thermo-regulation and cell infiltration in skin immunity.

Langerhans Cells: Ontogeny, Morphology and Function

Paul Langerhans, in 1868, described a peculiar looking cell in humans having dendritic processes in the suprabasal region of epidermis on gold impregnation. He and

the other researchers believed that these cells should be classified as a part of the nervous system (112). In 1926, Masson suggested that LCs were precursors of melanocytes (133). Only during the past three decades, the concept that LCs may have immune-related function was explored and accepted.

In the early 1960s, Breathnach and others showed that LCs were neither related to nerve cells nor to melanocytes (26). Only recently Katz demonstrated that LCs originate from a pool of bone marrow derived mobile progenitor cells (90). This observation was further reinforced by Frelinger et.al. when they illustrated synthesis of LCs in X-irradiated chimeric mice from bone-marrow derived donor cells (65). Precursors of LC were speculated to be of myeloid origin because they express myeloid surface molecules like CD11, CD13 and CD33 (69) and were presumed to immigrate to the skin through blood circulation. Transforming growth factor β1 (TGF β1) (22) has been demonstrated to be an essential requirement for *in vitro* development of $CD1a^+$, E-cadherin$^+$ and BG^+ LC from $CD34^+$ stem cells, under serum free conditions, in the presence of granulocyte macrophage-stimulating factor (GM-CSF), tumor necrosis factor α (TNFα), (31) and colony stimulating factor (CSF), while FLT-3 ligand was implicated in enhancing this development process (209). The complete set of signals needed for *in vivo* development and differentiation of LCs, from proliferating stem cell state to a precursor state, and intermediate stages involved in the development and the functional specialization of LCs, en route to skin are hardly known. Although a recent study suggested the involvement of GM-CSF, in the emigration of LC into the skin and in the maturation of LC, *in vivo* (29).

LCs exhibit a unique morphology. They have dendritic processes that interdigitate between keratinocytes and these processes can be seen with an aid of a light microscope. As mentioned previously, they are located in the basal and the suprabasal region of the

epidermis and make up 2 - 4% of the epidermal cell population. The most reliable marker that can only be visualized under electron microscope (78), in LC is the birbeck granule (BG). It is a rod shaped intracellular organelle speculated to have formed via an endocytic process (79) with an unknown function (18). Teunissen *et al.* have tentatively associated BG with antigen-trapping and antigen-presenting functions of LCs (218). The other classical markers used to identify LCs are Ia antigens, which are encoded by MHC II genes and membrane-bound ATPase (233, 63). These markers are unique to LC in normal epidermis and therefore are commonly used in immunochemical staining strategies. In case of skin disorders, KCs can be induced to express Ia antigen (10) or can acquire ATPase activity. In addition, LCs express CD1a in human epidermis (140), ly-5 antigens in mice (119) adhesion molecules, cytokine and Fc receptors for IgG and IgE (166, 115). The adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and leukocyte functional antigen 3 (LFA-3) (217) are weakly expressed on freshly isolated LCs though ICAM-3 is constitutively expressed (2). However, the mRNA for costimulatory molecules CD80 and CD86 is present in the cytoplasm of LCs, but it is not expressed on freshly isolated LCs from human epidermis (70, 210). Once LCs are cultured they resemble the mobile class of activated LCs found *in vivo*. Thus, the culturing of LCs stimulates LCs to undergo maturation and migration processes *in vitro*. The sets of changes that occur during this period are loss of BG, CD1a, $Fc\gamma R_{II}$ expressions (217), upregulation of MHC I, MHC II, ICAM-1, LFA-3 molecules and synthesis and expression of CD80 and CD86 (217, 216, 210, 165, 70). In brief, the phenotype or the morphology of LCs depends heavily on its milieu. LCs have a vast number of mitochondria indicating their high metabolic activity, well-developed

endoplasmic reticulum, golgi bodies and lysosomes (231). Their phagocytic capacity was found to be considerably less than that of macrophages and KCs (231).

The principal physiological functions of LCs in skin appear to be antigen recognition, antigen-uptake, antigen processing and selective migration towards T cell rich areas in order to present antigens to naïve T cells (203, 204,179). While performing all these functions LCs differentiate from an immature state to a mature state that finally culminates in a apoptotic death (134). LCs have also been implicated in contact hypersensitivity, skin-graft rejection, allergy and cutaneous immunologic tolerance.

Antigen presentation:

LCs similar to other DC's express pattern recognition receptors, complement receptors and Fc receptors that are involved in recognition and uptake of antigens. Studies suggest that LC express a mannose receptor that probably acts as an antigen receptor (161) and IgG receptors: CD32, CD64 and both high and, low affinity receptors for IgE (164) that allow for specific uptake of opsonized antigens. Once the antigen is engulfed via macro-pinocytosis or receptor mediated endocytosis (171), the next stage involves antigen processing. LCs belong to a professional class of antigen presenting cells that present exogenous antigen in conjunction with MHC class II molecules to $CD4^+$ T-cells (29). The MHC II molecule is synthesized and translocated to ER where it is associated with invariant chain. This complex is then moved to the endosomal compartment of the Golgi. The engulfed antigen is processed to a peptide form, which then binds with the cleaved MHC molecule in the MHC II compartment (MIIC), and is subsequently transported to the cell surface (145). This is a very important event because neither MHC II nor antigen individually can activate unprimed T cells due to the high

specificity of T cell receptor (TCR) for antigen. Lanzavecchia and others noted that efficiency of antigen uptake is crucial for peptide loading and presentation by MHC class II (113). In the normal unperturbed tissue, LCs have a strong antigen capture capacity but low APC activity hence they are termed as immature. Upon activation, LCs undergo a process of differentiation that triggers changes in the level of expression and repertoire of cell surface molecules. During *in vitro* cultivation of LCs for 2-3 days, differentiation signals are obtained from their microenvironments that in turn induce the maturation of LCs into potent immunostimulatory cells. These signals can be in the form of cytokines such as GM-CSF, IL-1β, IL-1α and TNFα (81). Once LCs are activated, they acquire a high level expression of MHC II, ICAM-1, ICAM-3, LFA-3, CD80, CD86, CD40 and ability to produce IL-1, IL-6, IL-12 and IL-15 (70, 216, 217, 198). Some of them serve to trigger migration of LC from the cutaneous region to peripheral lymph nodes and some to initiate T cell responses. In particular, TNFα and IL-1β have been demonstrated to cause LC migration (41, 43) as well as downregulation of E-cadherin that cements LCs to the neighboring KCs (183). Besides these, two other molecules implicated in LC migration are CD44 and integrin chains a6/b1 (156, 225). On reaching the lymph node, the antigen-MHC II complex on LC physically interacts with the TCR-CD3 complex on T-cells essentially delivering one signal while the binding of CD80/CD86 with their ligand CD28 on T-cells delivers a second signal for activation (180). Induction of a cascade of cytokines by activated T-cells, followed by upregulation of homing receptors on T-cells paves the way for their recruitment into skin (155) and a full-blown cell-mediated response.

Contact hypersensitivity:

LCs play a dominant role in the induction of T-cell activation in response to contact sensitizers. The proof that LCs serve as target structures in contact hypersensitivity type IV (CHS IV) reactions in humans on exposure to DNCB was gathered by Silberberg in 1976 (188). Since then other investigators have shown induction of delayed hypersensitivity reactions on exposure to haptens in a high LC density area whereas in a LC deficient area the same hapten application induced unresponsiveness or peripheral tolerance (219). Haptens are small chemical molecules that bind to a protein carrier, which confers immunogenicity. When the haptens are painted on the skin of sensitized individuals, the LCs gradually disappear from the epidermis, migrate to the draining lymph node, activate $CD4^+$ T cells, which then selectively migrate to skin, causing a local intracutaneous inflammation characterized by the presence of erythema and swelling (103, 106, 189, 217) as seen in the case of contact dermatitis. Although the mechanism of triggering and controlling the migration of LC is unclear, some recent studies indicate that caspase1 may play a role (7). It has also been speculated that certain cytokines like TNFα, IL-1β (59), secreted by KCs following hapten painting provide signals for LC migration. Adhesion molecules such as ICAM-1 are believed to play a central role since anti-ICAM-1 moAb blocked the APC function of EC in a hapten-painted mice (142).

Allergy:

Allergens may cause inflammation via triggering accumulation of immunocompetent cells in dermis and epidermis, giving rise to specific clinical manifestations such as atopic dermatitis. Studies indicated the presence of the high affinity receptor for IgE (FcεRI) on LCs in patients with atopic dermatitis (27). LCs with

these receptors apparently present the airborne allergens such as mites and pollen to specific Th2 subtype-cells which in turn may trigger a cascade of events resulting the activation of mast cells and basophils (28, 224). It was recently shown that IL-4 plays a key role in upregulation of FcεRI on human $CD1a^+$ LCs in atopic dermatitis patients (67). It has also been suggested that protein kinase Cβ (PKC) may provide a signal for LC mobilization in allergic and in irritant contact dermatitis cases (163).

Skin graft rejections:

In the murine system it was demonstrated that LCs induce allogeneic epidermal-cell-lymphocyte reaction indicating their involvement in allograft rejection and the graft-versus-host reaction (1) and it was also demonstrated that murine grafts depleted of LCs survived longer on I-region-disparate hosts (206). A study by Larsen et. al. suggests that epidermal LCs migrate from skin to afferent lymphatics and initiate transplantation rejection (117). The upregulation of cytokine IL-10 and lowered expression of MHC II molecules have been held responsible for the tolerogenecity of neonatal skin grafts (48). In organ systems like the pancreas, elimination of Ia^+ dendritic cells (DCs) facilitated acceptance of pancreatic grafts in rats (61). In addition to decreased MHC II expression, decreased B7 costimulatory molecule expression in photodyanamic-treated mice led to prolonged graft survival (147).

Immunological tolerance:

LCs play a decisive role in inducing immune responses as well as establishing tolerance. The knowledge concerning either immunogenic properties of LC or the factors that transform LC to be tolerogenic can be exploited to design vaccines against

neoplasms and micro-organisms, to control allergies, to treat skin disorders and to prolong graft survival. One of the extraneous factors responsible for hyporesponsiveness is UVB radiation. Dandie *et al.* observed in mice that even after two weeks after exposure, LCs were not capable of functioning normally (45). UVB irradiation has been shown to deplete the epidermal LC population (16). When these areas were exposed to contact sensitizers, CHS was not induced (219). This led to the belief that the ability of the skin to support the induction of CHS or specific unresponsiveness was influenced by the presence of functionally intact LCs (207). It was shown that suppressor T cells were responsible for induction of tolerance *in vivo* (226). Cytokines IL-10 (58), TGF-β (208), neuropeptides (98), α-melanocyte stimulating hormone (126), lowered B7 expression (101) have been implicated in establishing DC tolerance and T cell anergy.

Epidermal Cytokines and Chemokines

The emigration of LCs from skin to dermal lymph nodes is essential to mount a cell-mediated response and hence immune surveillance. Prior to migration, LCs have to become functional antigen presenting cells. This activation and the imminent migration of LCs are initiated and regulated in part by KCs derived inflammatory mediators called cytokines. Cytokines are low molecular weight proteins that are produced in a tightly controlled manner, by various cell types and act in both paracrine and autocrine fashion (8) as soluble mediators of immunity, inflammation, cell growth and differentiation in either synergistic or antagonistic way (127). The first cytokine discovered in 1976, interleukin 2 (IL-2), promoted *in vitro* T cell growth (139). Later it was discovered that

cytokines represent a wide array of different molecules with overlapping biological activities: hence, their function is considered as pleiotropic and redundant. These features emphasize the importance of cytokines in cellular function. Cytokines can induce expression of other cytokines (52, 91), interact with other mediators such as hormones, and bring about a cascade of events. Epidermis has been termed as a "cytokine factory", having producer and responder cells as well as the cytokines themselves responsible for skin disorders including contact allergy, atopic eczema, psoriasis etc. (56, 213). Some of the constitutive and inducible epidermal cytokines are IL-1α, IL-1β, IL-6, IL-10, IL-12, GM-CSF, TNFα and interferon γ (IFNγ). The cytokines IL-1α, TNFα and GM-CSF will be dealt in detail below.

Chemokines belong to a family of cytokines with cell-specific chemotactic activity. The three sub families of chemokines, α-chemokines or C-X-C chemokines, β-chemokines or C-C chemokines and γ-chemokines or C-chemokines are differentiated based on their protein sequence and function. The members of α-chemokine are chemotactic to basophils, lymphocytes and keratinocytes and are mostly produced by KCs in the epidermis (21). The members of β-chemokines subfamily, MIP-1α, MCP-1, MCP-3, RANTES and MDC are chemotactic for macrophages/ monocytes, T cells and DCs (12, 71, 193, 235). MCP-3 and RANTES are produced by KCs on challenge with allergen (237) and are potent attractants for eosinophils; T cells macrophages and APCs (178, 193). MIP-3α has been shown to play a role in LC precursor recruitment into the epithelium during inflammation (49) while MCP-1 does the same in the absence of inflammation too (141). The stimuli for regulation of chemokine expression are provided by cytokines such as IL-1β and TNFα (72) as well as contact sensitizers, tolerogens and irritants (138).

IL-1:

The IL-1 triad that includes IL-1α, IL-1β and IL-1RA (receptor antagonist) are 17-20 kDa proteins mainly involved in the initiation and maintenance of inflammation. Originally named as epidermal cell thymocyte activating factor (ETAF), IL-1 was the first cytokine detected in epidermal cells (128). IL-1 transcription in monocytes was induced by adhesion to surfaces, lipopolysaccaride (LPS), *S. aureus*, IL-1 itself, phorbol esters, zymosan, TNFα, IFNγ, Calcium ionophores, C5a, indomethacin and GM-CSF (46, 62, 82). IL-1α and IL-1β show similar biological activities since they bind signal transducing type I receptor with similar affinity (53). Some of the responder cells in which IL-1 induces proliferation are fibroblasts, smooth muscle cells (158), keratinocytes, hematopoetic precursors, Th2 cells and mature B cells (50). The other biological implications of IL-1 include induction of other cytokines expression such as IL-6, TNFα, GM-CSF from KCs; induction of TNFα and adhesion molecules on endothelial cells; induction of acute phase protein in hepatocytes; differentiation and surface IgM expression on pre-B cells; and immunoglobulin secretion from mature B cells; IL-2R expression and chemotaxis of T cells; and augmentation of macrophage cytotoxic activity. IL-1 plays physiological roles that influence pathological conditions such as fever, cachexia, wasting, septic shock, rheumatoid arthritis and atherosclerosis (51). Some of these observations are supported by the fact that the inhibitors of IL-1 are capable of suppressing such responses *in vivo* (60).

In epidermis, as mentioned earlier, KCs produce constitutively IL-1α, a cytokine essential for peripheral T cell activation. LCs are induced to produce IL-1β upon skin sensitization (59). IL-1β is important in the context of LC migration and cytokine upregulation (57).

GM-CSF:

GM-CSF is a 20-30-kDa glycoprotein named after the ability to induce proliferation and maturation of granulocytes and macrophages. Bacterial endotoxins, phorbol esters, TNFα, IL-1α and macrophage activating proteins can induce it. GM-CSF is a polyfunctional regulator that affects the growth and function of KCs, DCs, endothelial cells, T cells; increases DNA synthesis in transformed cells; enhances neutrophil chemotaxis; and augments the tumoricidal and the anti-microbicidal activity of neutrophils and macrophages (167). Excessive levels of GM-CSF can lead to blindness and muscle wasting in mice (111) and mild flu-like symptoms, rashes, capillary-leak syndrome and respiratory distress in patients (215).

In the skin, GM-CSF production has been observed both at the mRNA as well as at the protein level in fibroblasts, mast cells, macrophages and endothelial cells and in KCs it is induced upon reception of stimuli such as antigen, allergen, irritant, IL-1, UV-irradiation or inflammation (228). It was found to be important for LC viability *in vitro* (230), LC generation from precursors, antigen-presenting ability of DCs (86) and LC migration (168). Because of its potent immune-enhancing properties, is being exploited as a vaccine adjuvant in flu-vaccine, tumor vaccine, hepatitis B vaccine and HIV vaccine (211).

TNFα:

TNF is a 17-kDa protein originally described as macrophage-derived cytokine and also is known as cachetin and necrosin because of its biological effects. Macrophages, NK cells, T cells, B cells, granulocytes, fibroblasts, mast cells, smooth muscle cells, epidermal cells and tumor cells primarily produce TNF. It is produced in response to both

gram-positive and gram-negative bacteria and their products; UV-radiation; mycoplasma; immune complexes; cytokines such as GM-CSF, IL-1β, IL-2, IFNγ; tumor cells, C5a; PKC activators; protein phosphatase inhibitors; platelet aggregating factor (PAF) among others. TNF displays a broad spectrum of effects, which includes inhibition of tumor cells hence its therapeutic application as anti-tumor agent, proliferation of fibroblasts, expression of adhesion molecules on endothelial cells, induction of cytokines, induction of MHC expression on various cells. It exhibits peculiarly contradictory effects such as viricidal effects against DNA and RNA viruses and induction of viral replication (234, 148). TNFα was demonstrated *In vitro,* to activate phagocytes to kill bacteria; fungi; protozoa and helminths (229).

In epidermis, the expression of TNFα by keratinocytes is upregulated by allergens, LPS and UV-radiation (102). In cutaneous immunity it exerts its influence by increasing production of IL-1α by KCs (110), causing migration of LCs from epidermis (31), infiltration of macrophages, neutrophils and $CD4^+$ T cells in dermis and generating apoptotic KCs (181). It acts with GM-CSF synergistically to generate LC from $CD34^+$ bone marrow precursors (30).

Costimulatory Molecules: CD80 and CD86

LCs, like other DCs are critical for induction of primary immune responses and constitution of immunological memory (199). Following tissue damage or perturbation of normal skin milieu, LCs undergo a learning process, where they transform from a powerful antigen capturing state to an even more powerful antigen presenting state. One

of the events that co-ordinates tightly with the maturation process is the upregulation of costimulatory molecules such as CD40, CD58, CD80 and CD86 on LCs (179). The maturation of LCs is closely associated with its movement from epidermis to dermal lymph nodes and eventual priming of quiescent $CD4^+$ T lymphocytes which, is closely linked with expression of Ia antigen (186); CD80 and CD86 (94, 130, 69, 5); ICAM-1 and LFA-3 (190, 217) on APC. CD80/CD86 expression is predominant over expression of other costimulatory molecules on mouse dendritic cells (83). CD80 and CD86, integral membrane proteins, also known as B7-1 and B7-2 molecules respectively (99), are members of immunoglobulin superfamily that participate in T cell costimulation via CD28 and cytotoxic T lymphocyte antigen 4 (CTLA-4) ligands, surface proteins, on T cells (176, 179, 230). Witmer-Pack and others have demonstrated that in the absence of costimulation by CD80/CD86, TCR engagement is incapable of delivering effective signal in inducing T cell activation and instead induces anergy (179, 230). This specifically shows that the TCR-Ag+MHC complex provides one stimulus for T cell activation while CD80/ CD86 crosslinking with CD28 provides the other stimulus (176, 222). This enhances T cell survival by augmenting expression of Bcl XL, an inhibitor of apoptosis (20). The engagement of CD80/CD86 with CTLA-4 on T-cells leads to the inhibition of T cell activation, validated by the fact that a CTLA-4 null strain of mice exhibits severe lympho-proliferative disorder (184) and exposure to CTLA-4-Ig renders DCs tolerogenic (94, 124). Some of the mechanisms that were reported to account for the action of CTLA-4 include induction of apoptosis (73), cell cycle arrest (175) and establishment of an effective threshold for activation of T cells (93, 107, 122, 223).

CD80 and CD86 are differentially expressed and regulated on LCs in response to cytokines (92). Though the molecules exhibit some functional redundancy, in cases like

determining Th1 response versus Th2 response, they show distinct effects (64, 108). A study conducted to demonstrate differential Th1/Th2 activation pathway showed that IFNγ and IL-10 down regulate Th1 clonal expansion by suppressing CD80 expression (149). CD80 expression is positively regulated by cytokines TNFα, GM-CSF but not IFNγ and IL-10; LPS; CD40L feedback (149, 170); by contact sensitizers (146, 162) on LCs while CD86 expression is positively regulated by few of the same mediators and also IL-4, but not TNFα and IL-10 (135). Recent findings showed that CD80 and CD86 mediate costimulatory functions with different magnitude. Expression of CD80 was reported to have increased the sensitivity of TCR for low affinity ligands (238). Transgenic mice expressing CD80 on KCs, a ligand normally absent on these cells, showed enhanced response to topical antigen, underlying its functional significance (227). Although recent studies indicate that CD86 is the major player in costimulation (159, 211). It was implicated in inducing allogeneic T cell responses as well as Ag-induced T cell proliferation (159). Though the mechanism of action is not clear, it is hypothesized that functional attributes of CD80/CD86 are due to decreasing the proximity, and facilitating signaling and lowering the threshold of activation.

Blocking of B7-CD28 interaction represents a powerful immunosuppressive tool to control allograft rejections by maximizing tolerogenic signals (120).

Adhesion Molecules: ICAM-1 and LFA-1

As mentioned previously, adhesion molecules aid in selective leukocyte migration to specific sites during immune responses by interacting with endothelium and aid in the

costimulation of resting T cells (131, 195, 38, 45). Three classes of adhesion molecules are known: the selectins, the integrins and the Ig supergene family (195). DCs including LCs, express a variety of adhesion molecules. Some of them are ICAM-1, ICAM-2, ICAM-3, E-cadherin, E-selectin and V-CAM. These molecules exhibit selective expression and regulation patterns.

In the epidermis, KCs and LCs show the expression of homophilic adhesion molecules called E-cadherin that acts like cement maintaining the cellular contact between the two cells (214). Loss of E-cadherin was found to be linked with accumulation of DCs in lymph nodes (22). Allergens and cytokines such as TNFα and IL-1β have been to some extent implicated in the downregulation of these molecules (183). Another molecule that is important in LC migration is ICAM-1 (27). ICAM-1 is a 95-kDa member of the Ig superfamily, also known as CD54, found on leukocytes, endothelial cells and DCs among others (143). It interacts with its ligand LFA-1, known as CD11a/CD18, a heterodimer surface molecule expressed on all leukocytes (118, 197) and particularly endothelial cells in the epidermis. It modulates intercellular adhesions (118, 196) as well as costimulatory effects (109). Antibodies against both these molecules have been shown to prolong allograft survival (80, 85, 221), reduce the severity of autoimmune response (104), and inhibit contact hypersensitivity (129) by blocking leukocyte migration (167). ICAM-1 was shown to have a non-MHC restricted effect on tumor cells (143, 144). A synergistic effect was observed between CD80 and ICAM-1, in delivering a more intense second signal for T cell triggering in artificially constructed drosophila cells (194). T cells also possess adhesion molecules LFA-2 and ICAM-3 that bind with LFA-3 and LFA-1 on DCs and cause upregulation of costimulatory and other functional molecules (157).

Maturation and Migration of Langerhans Cells

The strategic location of LCs on the skin surface makes them ideal for monitoring invasion by extraneous factors and protecting the integrity of epithelial surfaces from these factors. They evolved to perform the role of antigen presenting cells to dormant T cells. The sequences of steps involved in the life cycle of LC starting from its recruitment as APC are: capturing exogenous antigens, processing them to small peptides and complexing them up with MHC II moieties, exporting this epitopic complex on to the surface, upregulating expression of various costimulatory molecules, downregulating expression of other molecules such as E-cadherin, undergoing changes in the cell morphology: depolarization, appearance of filopods, cytoskeletal rearrangement, binding to Laminin, regulating access across basement membrane, accumulating in lymphatics, engaging specific surface structures with their respective ligands on T cells, bringing about activation of T cells and undergoing apoptosis. All these events form an inseparable part of a maturation and migration process of LCs and are intricately governed by the changing cytokine scenario.

In the epidermis, LCs are maintained at a specific density (15, 233) and their turn over rate is low (121, 205). This implies that LCs are not native residents of epidermis and have immigrated from other organ/organ systems. This was proved by Katz and others that LCs predecessors are nomadic bone marrow-derived $CD34^+$ cells (90). *In vitro* culture of human cells, $CD34^+$ blood cells generated a subset of cells with CD1a, HLA-DR, CD86 and birbeck granules, in the presence of GM-CSF and TNFα (30).

Recently, common myeloid progenitors (CMP) were isolated (132). On transplantation in irradiated congenic recipients, these progenitors gave rise to LCs. The homeostasis of LC is believed to be regulated by cytokines. Though *in vivo* factors are not clear, it has been shown that GM-CSF, IL-1 and TNFα maintain LCs in viable state in culture (101).

After they develop from progenitors, LCs populate skin and remain in an unfledged state. They are induced to mature and migrate by a variety of stimuli including microbial antigens, inflammatory mediators, tissue damage, UV radiation and other antigen non-specific signals. *In vitro* culture of LCs triggers the maturation process and increases their immuno-stimulatory ability (230). Once the integrity of the skin is disturbed, LCs undergo phenotypic and functional changes that imitate changes that occur in cell cultures. Studies indicate that in skin grafts (118) on treatment with skin sensitizers (4), on exposure to enterotoxins (35) DCs show marked induction of Ia^+ antigens as it does on stimulus with GM-CSF, IL-1, TNFα and CD40 ligation (32, 171). IL-1β and TNFα are responsible for switching of LC from the Ag-capturing stage to the Ag-presenting state (172) as they are responsible for shut down of macropinocytosis. Keratinocytes produce increased levels of GM-CSF, TNFα and IL-1α expression in response to danger signals whereas LCs produced increase levels of IL-1β (59). Cytokines have been implicated in activation, maturation via upregulation of various surface molecules and migration of LCs both directly and indirectly by regulating cellular adhesions. Recent publication indicated that Cytosine-guanosine (CpG) motifs present in the bacteria DNA induce LC migration by modifying expression of E-cadherin and ICAM-1 (13). Reynolds and his co-workers have suggested based on their data that LC migration in the case of CHS involves the PKC-β signaling pathway (163). TNFα has

been demonstrated to produce subsequent effects that may possibly influence the maturation and migration process: reduction in the expression of E-cadherin *in situ* (183), an increase in the expression of ICAM-1 (42, 220), induction of the expression of gelatinase in macrophages (173), chemotaxis for mLC (236), and upregulation of expression of CD80 and CD40 (170). IL-1β induces expression of gelatinase, reduces expression of E-cadherin (183), induces expression of other cytokines such as TNFα, IL-1α, IL-10, MIP-2 (57), induce expression of chemokines (11) and thereby regulates both maturation and migration process. GM-CSF is another cytokine that modulates maturation and migration process of LCs by upregulating costimulatory molecule expression (170). IL-18 has been shown to induce LC migration by an IL-1 dependent mechanism (39).

The significant aspect of LC migration is that LCs do not need to be fully mature in a functional sense before they migrate from epidermis. This was demonstrated by Larsen *et al.* who showed that LC start migrating even before they acquire all the relevant maturation markers (117). Studies have indicated that LCs enter lymph nodes just after 4 hours exposure to FITC, reach a maximum after 2 days and gradually decline thereafter (130). As stated in the previous section, LCs start undergoing maturation in response to external stimuli, while at the same time acquiring molecules that regulates their trafficking from the epidermis. The two most important molecules on LCs that are involved in the exodus are E-cadherin and ICAM-1. Downregulation of E-cadherin and upregulation of ICAM-1 on LCs and upregulation of LFA-1 on endothelial cells aid in directional movement of LCs across the basement membrane. The three steps involved are: a, detachment of LCs from their neighbors, KCs by downregulating E-cadherin expression; b, Attachment of LCs and Endothelial cells via ICAM-1 and LFA-1 and

initiating the rolling movement that resembles molecular "Velcro"; and c, transendothelial translocation. The migration of LCs is important because the event is succeeded by the elicitation of antigenic-specific T cell responses that could have a range of implications for cutaneous immunity. The pathological importance of this event is supported by the fact that a murine retrovirus, Rauscher leukemia virus ensures its survival by blocking LC migration (66).

The factors that are responsible for LC migration are numerous and intricately linked together. Any strategy that tries to explain this must consider the complex interactions between various costimulatory molecules, adhesion molecules, cytokines, chemokines and cell populations. For instance, Kitayama *et al.* showed that ligation of CD80 with CD28 delivers a signal leading to the secretion of IL-1β (97). Cumberbatch *et al.* showed IL-1β as one of the main signals for LC migration (41). Thus it remains to be seen whether ligating CD80 promotes LC migration. The present study endeavors to examine the effect of antibody against CD80 and focuses on the role of cytokines in an anti-CD80 induced response.

CHAPTER II

MATERIALS AND METHODS

Animals

Young female BALB/c strain mice, 6-to-12 weeks old were obtained from Charles River (Wilmington, MA). These mice were maintained in the Animal Resources Laboratory, an approved facility at Oklahoma State University.

Media and Solutions

Phosphate buffered saline (PBS, 0.15M) was used as diluent for solutions of trypsin (type II from porcine pancreas, Sigma Chemical Company, St. Louis, MO), deoxyribonuclease I (DNase I, from bovine pancreas, Sigma Chemical Company, St. Louis, MO), antibodies and cytokines. One liter of 0.15 M PBS contains 1.15 g dibasic anhydrous sodium phosphate (Na_2HPO_4, Sigma Chemical Company, St. Louis, MO), 0.2 g potassium chloride (KCl, Fisher Scientific, Fairlawn, NJ), 0.2 g monobasic potassium phosphate (KH_2PO_4, E M Science, Gibbstown, NJ) and 8.0 g sodium chloride (NaCl, J. T. Baker Chemical Co., Phillipsburg, NJ) in 1000 mL deionized water and adjusted to pH 7.2 using 1 N solution of HCl before sterilization at 15 psi for 20 min.

PBS with Tween 20 (PBS Tween, Sigma Chemical Company, St. Louis, MO) was used as washing buffer and PBS with 10% Fetal Bovine Serum (FBS, Sigma Chemical Company, St. Louis, MO) was used as blocking buffer in immunohistochemical staining of epidermal skin section. In the same experiments, skin sections were floated on 2 M sodium bromide (NaBr, Fisher Scientific, Fairlawn, NJ) to separate epidermal and dermal layers.

Skin sections, epidermal cells suspensions were cultured in and cell washings were performed in sterile RPMI 1640 media (Sigma Chemical Company, St. Louis, MO) supplemented with 10% FBS (Sigma Chemical Company, St. Louis, MO), 100 μg/mL penicillin (Sigma Chemical Company, St. Louis, MO), 0.1 μg/mL streptomycin (Sigma Chemical Company, St. Louis, MO), 0.1 mM MEM non-essential amino acids (Sigma Chemical Company, St. Louis, MO), 2 mM L-glutamine (Sigma Chemical Company, St. Louis, MO) and 0.1 mM sodium pyruvate (Sigma Chemical Company, St. Louis, MO).

Antibodies

To stain MHC class II (Ia) cell surface antigen, encoded in the I-A region of H-2^d haplotype, on Langerhans cells, purified mouse anti-mouse-I-A^d monoclonal antibody (Pharmingen, San Diego, CA) of IgG isotype was used.

Purified monoclonal rat anti-mouse CD80 (B7-1) (Pharmingen, San Diego, CA), monoclonal rat anti-mouse GM-CSF (Pharmingen, San Diego, CA), monoclonal hamster anti-mouse ICAM-1/CD54 (Pharmingen, San Diego, CA), monoclonal hamster anti-mouse IL-1β (Genzyme, Cambridge, MA), monoclonal hamster anti-mouse IL-1α

(Genzyme, Cambridge, MA), monoclonal rat anti-mouse LFA-1/CD11a (Pharmingen, San Diego, CA), and monoclonal hamster anti-mouse TNFα (Pharmingen, San Diego, CA) antibodies were subcutaneously administered for neutralization. All antibodies were of the IgG isotype. PBS was used as negative control for *in vitro* and *in vivo* studies.

Cytokines

Recombinant murine granulocyte macrophage colony stimulating factor, recombinant murine tumor necrosis factor α and recombinant murine interleukin-1β (Genzyme, Cambridge, MA) were added to epidermal cell suspension before performing immunoassays for TNFα and GM-CSF.

Preparation of Epidermal Sections

Female Balb/c mice were injected intraperitoneally with neutralizing antibodies against CD80, GM-CSF, ICAM-1, IL-1α, IL-1β, LFA-1, and TNFα individually and concomitantly with anti-CD80 antibody. After a lapse of two hours in order to let the antibodies circulate throughout, the mice were euthanized in a CO_2 chamber. Hair from the dorsal side of the mice were removed initially by shaving and additionally by using an over-the-counter depilatory lotion (Nair™). After a thorough wash with sterile distilled water and 70% ethanol, the dorsal skin was surgically removed, the subcutaneous adipose tissue was scraped off and then the skin was cut into 1-cm^2 sections in a sterile laminar

flow hood. These skin sections were then placed epidermal side up on a stack of three sterile filter disks soaked with 600 μL of supplemented RPMI 1640 media in a Falcon ™ 24-well tissue culture plate. Following 48 hours of incubation at 37° C in the presence of 5% CO_2, the skin sections were floated dermal side down on 2 M sodium bromide for 45 minutes at 37° C to separate the epidermis from the dermis. Epidermis was gently pried off from the dermis with a scalpel in a petri dish of PBS. The epidermal sections were then stored in PBS at 4° C for future usage.

Immunocytochemical Staining

Epidermal sheets were fixed for staining in HistoChoice™ tissue fixative (Fisher) for 20 min at 4° C and subsequently washed thrice with PBS. The sheets were then incubated for 30 minutes at room temperature in 3% hydrogen peroxide to neutralize endogenous peroxidase activity. Afterwards they were washed three times with PBS-Tween 20, further incubated in fresh 3% hydrogen peroxide for an additional 20 minutes at room temperature. Sections were washed again with PBS-Tween and blocked with 10% FBS in PBS for 30 minutes at room temperature. After a final wash with PBS, epidermal sections were incubated at 4° C overnight with anti-mouse I-A^d antibody. After incubation with primary antibody, sections were washed and incubated for 35 minutes first with biotinylated anti-mouse IgG antibody from Vectastain™ elite mouse IgG ABC kit (Vector Laboratories Inc., Burlingame, CA) and followed with Vectastain ABC

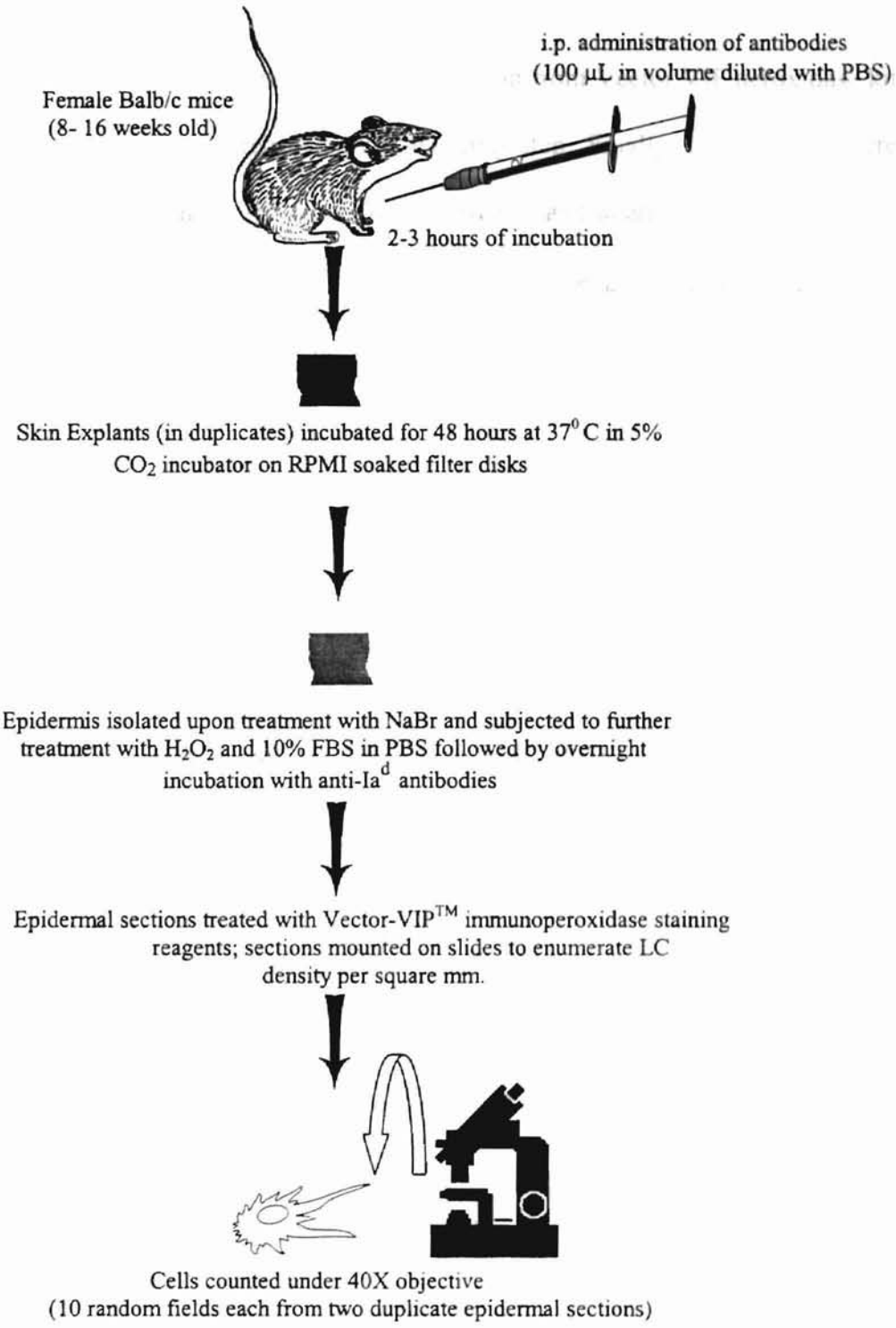

Figure 1. Flow Chart depicting Immunohistochemical Staining of Murine Epidermis. Ia^+ LCs are counted following treatment with antibodies specific against CD80 molecules, cytokines and adhesion molecules.

reagent and finally for 12 minutes with substrate solution from Vector VIP peroxidase kit (Vector). Each treatment was preceded by a washing step. Finally, the sections were rinsed with distilled water, mounted on a slide in glycerol and quantified for Langerhans cells with an aid of a microscope. The frequency of Ia^+ LCs (measured as cells/mm^2) was obtained by enumerating 10 random fields on two duplicate epidermal sections.

Epidermal Cell Suspensions

Each Balb/c mouse was sacrificed with ether and its hair removed using clippers and depilatory cream. After washing the mouse, the skin from dorsal and ventral sides, the ears and the tail were surgically sectioned out. In a sterile Laminar flow hood the subcutaneous layer was scraped out, the skin was cut into two or more manageable segments and incubated dermal side down in 0.5% trypsin for 45 minutes at 37° C. Following incubation, the epidermal layers were obtained by smoothing out skin sections with their epidermal sides down on a clean and a dry petri dish and jerking them gently to the side. Immediately the exposed basal layer of the epidermis was covered with 0.05 % DNase. The basal layer was removed from the tail region by using forceps and DNase was added as before. The samples were rubbed with a blunt-tip glass rod to bring the cells into suspension. Three volumes of RPMI 1640 medium were added to the suspension, aspirated approximately 15 times with a 60 cc syringe (Fisher, Fairlawn, NJ) and filtered through a Falcon 70 μm nylon mesh cell strainer (Becton Dickinson, Lincoln Park, NJ) into a Sarstedt 50 mL test tube (Sartedt, Princeton, NJ). The cells were then precipitated out in a Damon CRU-5000 centrifuge (IEC, Needham Heights, MA) for 8

minutes at 4° C, washed three times to remove all traces of DNase I, resuspended in 10 mL of media and quantified by adding 10 μL of cell suspension in trypan blue (Sigma, St. Louis, MO) exclusion to hemacytometer. The suspension was finally adjusted to obtain a desired cell concentration.

Cytokine Assays

To assay for TNFα and GM-SCF commercially available Quantikine™ mouse TNFα and Quantikine™ mouse GM-CSF kits (R&D Systems, Minneapolis, MN) were respectively used.

Epidermal cell suspension at a concentration of 1.0×10^6 cells/mL was plated out 1.0 mL/well in a 24-well tissue culture plate (Sigma), in duplicates, and stimulated with 50 μL of 10 μg/mL anti-CD80 antibody, 0.1 μg/mL IL-1, 0.01 μg/mL TNFα and 2 μg/mL GM-SCF separately and in combination with the former. Additionally 50 μL of 10 μg/mL anti-LFA-1 was added to a new well containing 1 mL of cell suspension. 0.15 M PBS was added to the control group and the plate was incubated at 37° C. Supernatants from each treatment group were directly analyzed for cytokine production after appropriate incubation times.

TNFα Immunoassay:

This assay employed a quantitative sandwich enzyme immunoassay principle where a 96-well microtiter plate was pre-coated with purified polyclonal antibody specific for mouse TNFα. After 24 hours of incubation, samples from various treatment groups were added in 50 μL volumes to the well and incubated for 2 hours at room temperature to

allow binding of mouse TNFα present in the samples to immobilized antibody. Unbound material was washed away and horseradish peroxidase conjugated anti-mouse Ig was added and the mixture was incubated as before. Following a wash to remove any unbound antibody-enzyme complexes, a substrate solution was added to the wells. The enzyme reaction subsequently yielded a blue product, which turned yellow on addition of stop solution. The optical density of each treatment group was read using an EL800 Universal Microplate Reader (Bio-Tek Instruments Inc., Winooski, VE) set to a wavelength of 450 nm. Standard curve for each set of samples was generated by plotting the mean absorbance value for each standard on the y-axis against the known values of concentration on the x-axis. Then the sample values were read off the standard curve in pg/mL.

GM-CSF Immunoassay:

This assay employed a quantitative sandwich enzyme immunoassay principle where a 96-well microtiter plate was precoated with affinity purified polyclonal antibody specific for mouse GM-CSF. After 48 hours of incubation, samples from various treatment groups were added in 50 μL volumes to the wells and incubated for 2 hours at room temperature to allow binding of mouse GM-CSF present in the samples to immobilized antibody. Unbound material was washed away and horseradish peroxidase conjugated anti-mouse antibody was added and the mixture was incubated as before. Following a wash to remove any unbound antibody-enzyme complexes, a substrate solution was added to the wells. The enzyme reaction yielded a blue product, which turned yellow on addition of stop solution. The optical density of each treatment group was read using an EL800 Universal Microplate Reader (Bio-Tek Instruments Inc.,

Winooski, VE) set to a wavelength of 450 nm. The sample values were then obtained in pg/mL from the standard curve generated by linear regression analysis of standard GM-CSF concentrations versus absorbance values.

Indirect Immunofluorescence

Each epidermal cell suspensions were adjusted to 2 x 10^7 cells/mL and 50 µL of this was aliquoted in a 96-well tissue culture plates. The suspensions were then treated with 10 µg/mL of TNFα and 2 µg/mL of GM-CSF separately and in combination with each other and incubated for 48 hours at 37^0 C. The cells were washed twice in 2% FBS in PBS, centrifuged for 7 min at 1700 rpm and fixed. Cells were incubated on ice with 10 µg/mL of anti-CD80 antibodies for 43 minutes. Washed and centrifuged, the cell precipitates were then incubated in ice with anti-rat IgG FITC for 40 minutes. After a final wash and centrifugation step, the cells were resuspended in 2 drops of 90% glycerol in PBS and mounted on a slide. The cells positive for CD80 were counted using a fluorescent microscope and their percentage was calculated.

CHAPTER III

RESULTS

The primary aim of this study was to investigate the role of CD80 in the induction of Langerhans cell (LC) migration in a murine system and its dependence upon pro-inflammatory cytokines. The effect of cytokines GM-CSF, IL-1α, IL-1β and TNFα on LC migration has already been elucidated to a certain extent, but not their impact on anti-CD80 antibody induced LC depletion. Hence, this project also focuses on the latter aspect of anti-CD80 induced LC migration. All the data recorded were pooled from three experiments for each investigation and expressed in terms of the standard error of the mean (SEM). The Student t-test was performed to ascertain the statistical significance of the results. In addition, Dunnetts t-test was used to establish the difference between the mean of the control group and the means obtained from various treatments.

Effects of Antibody against CD80 on *In Vitro* LC depletion

CD80 and CD86 are the chief costimulatory molecules that are differentially expressed and regulated on Langerhans cells (92). These molecules are involved in activation of primary resting T cells by providing one signal for the activation while crosslinking of MHC II-Ag complex with TCR provides the other signal. Interest in the role of CD80 in LC migration was sparked by a chance demonstration of depletion in LC number in epidermis on treatment of a mouse with neutralizing antibody against the

CD80 molecule. Hence a series of experiments were performed to examine the influence of these antibodies on LC migration from epidermis.

For this purpose epidermis was harvested and stained for IA^+ cells via immunoperoxidase staining two hours after intraperitoneal administration of Anti-CD80 antibody. Figure 2 indicates that 50 μg/mL of anti-CD80 antibody caused a decrease in the density of LC, determined as the mean number of LC per square millimeter of epidermis in two representative experiments. In another independent research we tried to determine the dosage effect of anti-CD80 antibody (100 g/mL and 200 g/mL) as well the effect of incubation time (24 hours) on LC depletion (data not shown). We found no substantial difference either due to the increase of antibody concentration or decrease of incubation period. The controls were from mice that were not treated with any antibodies and were observed to be with in 800-1000/mm^2 range mostly, consistent with experiments performed by others (42). A Student t-test was performed to establish a difference between control and experimental groups and this difference was found to be statistically insignificant. The results from the Dunnett's test emulated basically the results of the Student's t-test. Indirect evidence to ascertain if the decrease observed involved migration of LC in response to antibody against CD80 was obtained when antibodies administered against both ICAM-1 and LFA-1 adhesion molecules were shown to have blocked the depletion as depicted in the Figure 2.

This treatment was chosen to verify LC migration due to the fact that other investigators have found that successful accumulation of LC in draining lymph nodes occurs on upregulation of ICAM-1 expression (44) and also subcutaneous administration of antibody to ICAM-1 and LFA-1 led to a complete inhibition of contact hypersensitivity (129). The results also indicate that antibody to ICAM-1 produced a

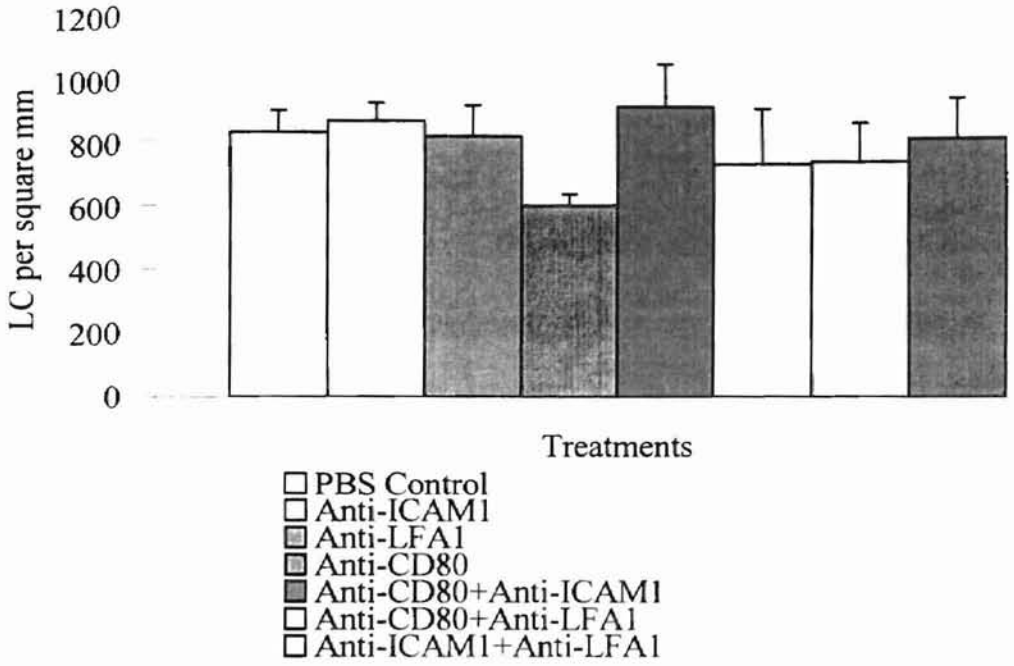

Figure 2. *In vitro* blocking of Anti-CD80 induced LC depletion by Anti-ICAM1 and Anti-LFA 1 Antibodies. After 2-4 hours on i.p. administration of antibodies, skin sections were dissected out and incubated on RPMI soaked filter for 48 hours. Epidermal sheets were then isolated and prepared for immunoperoxidase staining with anti-MHC class II antibodies. The density of Langerhans cells was determined by counting 10 random fields per sheet (2 duplicate sheets per experiment). The results are expressed as mean +/- of the SEM from two independent experiments (i.e., 2 mice per treatment group). Statistically significant difference in the means of the control group and the other groups treated with various antibodies are indicated by an *.

more pronounced inhibitory effect when compared to treatment with antibody to LFA-1. No synergistic effect was established as a result of co-administration of anti-ICAM-1 and anti-LFA-1 antibodies. Again, the Student t-test showed no significant difference between the various treatment groups. Nevertheless, these results demonstrate that there is likelihood that anti-CD80 antibody causes LC migration though quite small in magnitude and this mechanism is in part, dependent upon upregulation of adhesion molecules ICAM-1 and LFA-1. Another observation of note in this experiment is that anti-LFA 1 specific antibody which belongs to the same isotype class as anti-CD80 (IgG2a) does not cause depletion in LC frequency. This is important because in this study no IgG controls were used to rule out the involvement of Fc receptors.

Effects of inhibiting LC depletion by Anti-CD80 Antibodies using Anti-GM-CSF, Anti-IL-1α, Anti-IL-1β and Anti-TNFα Antibodies

As previously mentioned, studies have been performed to explain the role of cytokines in regulating LC migration in response to haptens. Evidence gathered from these studies indicates that TNFα and IL-1β play a significant part in mobilizing LC from epidermis to dermal lymph nodes (41) while GM-CSF and IL-1α were implicated in the process also (168, 185). Therefore our investigations focussed on these cytokines to determine if they were the candidate cytokines that regulated the anti-CD80 induced effect on LC migration. The antibodies to these cytokines were injected in combination with anti-CD80 in Balb/c mice intraperitoneally. The skins were harvested and incubated for 48 hours at 37% in a 5% CO_2 incubator. The epidermis was then isolated and stained for I-A^d surface antigen by the peroxidase Vector VIP reaction mixture.

The results indicated that simultaneous injection of 100 μl of 100 μg/mL anti-TNFα antibody blocked the anti-CD80 induced LC depletion as assessed by the frequency of Ia+ cells (Figure 3). The anti-CD80 induced effect was statistically significant at the 95% confidence level as established by both Student's t-test and Dunnett's t-test. Also of interest in this experiment was the observation that a ~27% inhibition of anti-CD80-induced decrease was achieved with anti-TNF antibody. In Figure 4, it can be noted that 100 μL of 100 μg/mL of neutralizing antibody against IL-1β partially blocked anti-CD80 induced depletion effect. The relatively less significant effect of anti-IL-1β can be explained based on the indirect nature of IL-1β's effect on LC migration suggested by the work of others (41). The current theory is that IL-1β interacts with keratinocytes to trigger production of TNFα. But another possibility is that the culture media might have been contaminated with LPS, a factor with known effect on LC depletion. The decrease in LC number on treatment with IL-1β specific antibody also supports this possibility (Fig. 4). Treatment with the 100 μg/mL antibody against IL-1α was without effect on the influx of LC as depicted in Figure 5 while 100 μg/mL antibody against GM-CSF produced ambiguous results. Interestingly again anti-IL-1α seemed to have caused some depletion on its own. A peculiar aspect of this experimental set up was that the controls were much below the range (800-1000/mm^2). This could loosely be attributed to the possible LPS contamination of the media, as in previous case, or to the age of mice used in these series of experiments. Some researchers in the past have established a direct relationship between the number of LCs residing in the skin and the age of the organism. In Figure 6, it is illustrated that antibody against GM-CSF on its own caused some depletion in LC number.

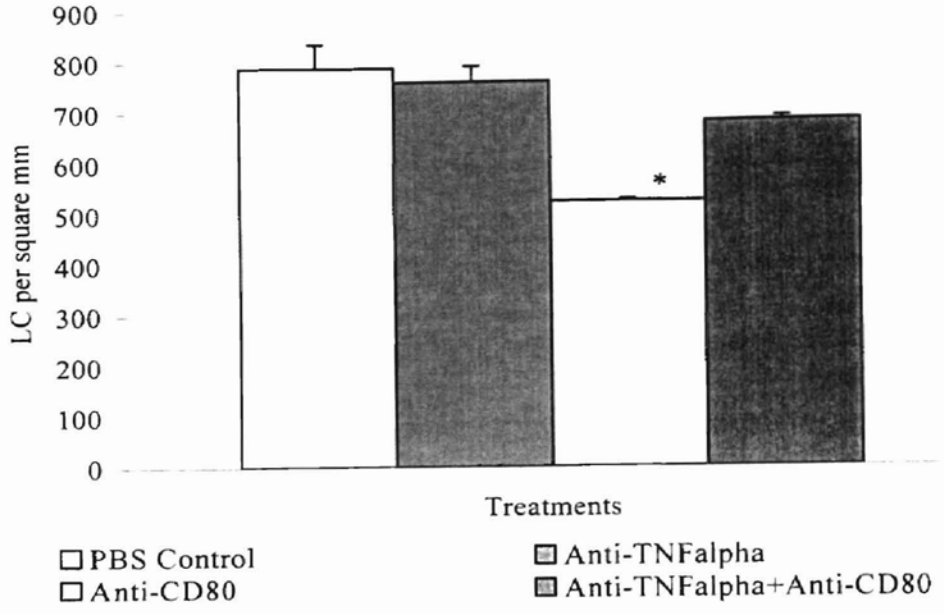

Figure 3. *In vitro* blocking of Anti-CD80 induced LC depletion by Anti-TNF Antibodies. Pretreatments of mice with antibodies are same as described in the legend to Fig. 1. The results are expressed as mean +/- of the SEM from three independent experiments (i.e., 3 mice per treatment group). Statistically significant difference in the means of the control group and the other groups treated with various antibodies are indicated by an *.

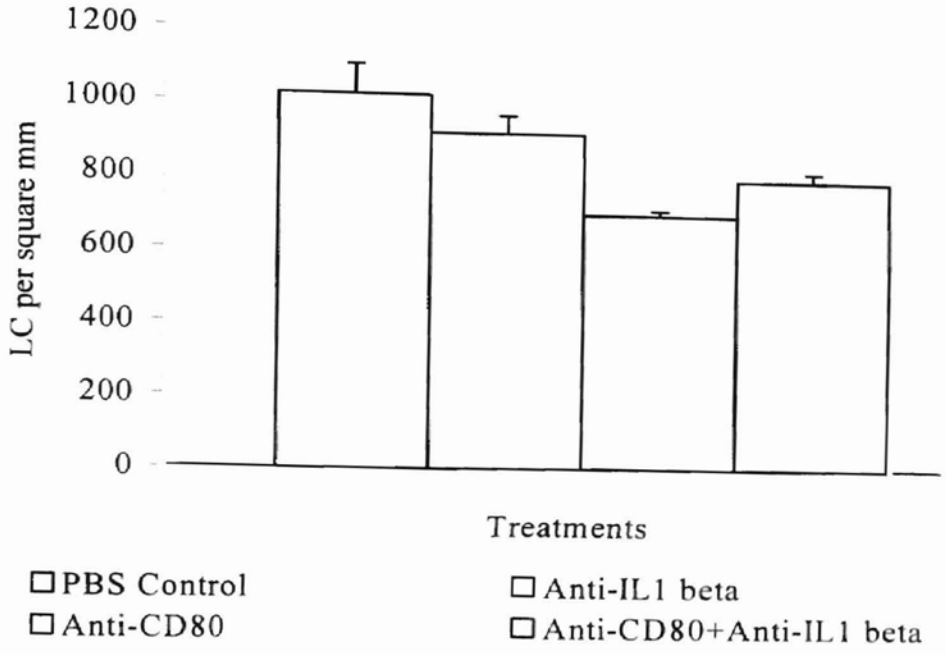

Figure 4. *In vitro* blocking of Anti-CD80 induced LC depletion with Anti-IL1 beta. Pretreatments of mice with antibodies are same as described in the legend to Fig. 1. The results were generated from two experiments as the mean +/- of the SEM. Statistically significant difference in the means of the control group and the other groups treated with various antibodies are indicated by an *.

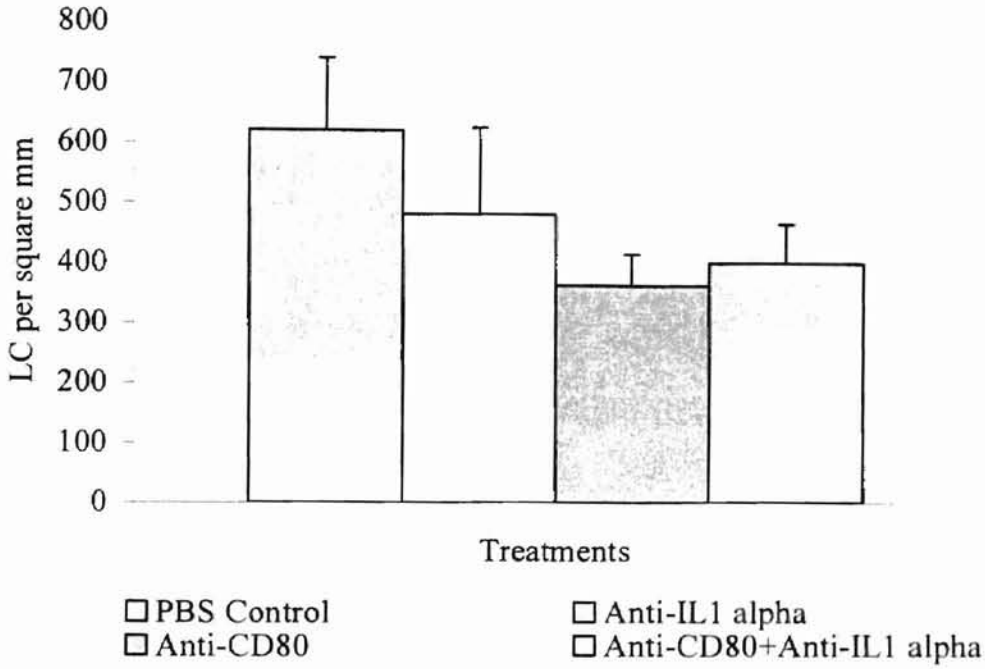

Figure 5. *In vitro* blocking of Anti-CD80 induced LC depletion with Anti-IL1 alpha antibody. Pretreatments of mice with antibodies are same as described in the legend to Fig. 1. The results were obtained as mean +/- of the SEM from two independent experiments. Statistically significant difference in the means of the control group and the other groups treated with various antibodies are indicated by an *.

To explain this the LPS induced depletion theory could be revoked but few other possibilities are discussed with in the chapter IV. When administered concomitantly with anti-CD80 antibody it blocked the effect of anti-CD80 antibody induced LC depletion but to a lesser extent. The Student's t-test and Dunnett's t-test showed a statistically insignificant outcome for the anti-IL-1α and the anti-GM-CSF-induced effects.

Overall, these results implicate TNFα as a primary mediator of anti-CD80 induced depletion and GM-CSF as a secondary mediator due to its less pronounced effect.

Cytokine Secretion by Epidermal Cells

One of the important questions to be addressed was whether cytokines play an upstream or a downstream role in anti-CD80 induced depletion effect. Earlier studies demonstrated cytokine production by epidermal cell populations. Hence, immunoassays were performed to analyze the secretion of TNFα and GM-CSF by epidermal cells on treatment with anti-CD80 antibody. Whole epidermal cell suspensions at a concentration of 1 x 10^6 cells/mL were cultured for 24 hours at 37°C in the presence TNFα, IL-1β, GM-CSF separately and in combination with anti-CD80 antibody to determine the levels of TNFα secreted using Quantikine™ murine TNF immunoassay kit (data not shown). The data obtained from two such experiments does not demonstrate that TNFα expression was induced exclusively as a result of anti-CD80 treatment to any significant level. On the contrary, control and the groups treated with IL-1, GM-CSF individually and in combination with anti-CD80 also showed comparable levels of TNFα production

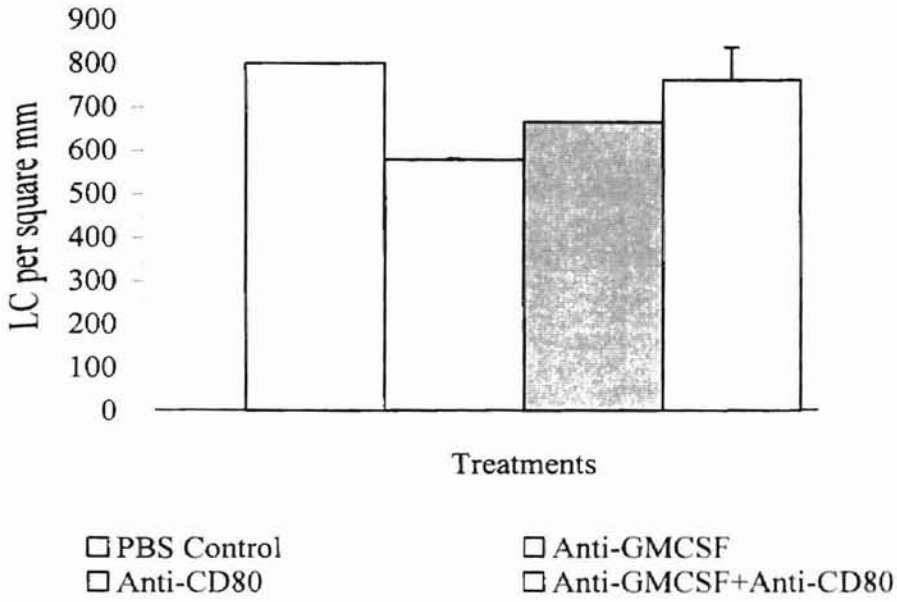

Figure 6. *In vitro* blocking of Anti-CD80 induced depletion by Anti-GM-CSF Antibodies. Pretreatments of mice with antibodies are same as described in the legend to Fig. 1. The results are expressed as the mean +/- of the SEM obtained from two experiments. Statistically significant difference in the means of the control group and the other groups treated with various antibodies are indicated by an *.

which were in the range of 200 pg/mL. The experiment was repeated with 48 hours time point with out any significant change in the level of expression of TNFα. In addition to TNFα, whole epidermal suspensions were exposed to the same set of cytokines for 48 hours and assayed for GM-CSF production in three separate experiments using Quantikine™ murine GM-CSF kit. The results again indicated that there was negligible difference in the levels of GM-CSF induced as a result of anti-CD80 treatment when compared with either control group or other treatments. The GM-CSF concentration was approximately in the range of 220 to 250 pg/mL in all cases except when the treatment involved exposure to GM-CSF itself in which case it was 500 pg/mL, presumably due to the presence of residual cytokine. The results of this study show that the cytokine production by anti-CD80 may not possibly explain the anti-CD80 effect. Thus, it is more likely that cytokine upregulation of CD80 expression; GM-CSF (116) and TNFα in particular, may explain anti-CD80 induced mobilization of LC as depicted in the Figure 6. The data presented in the Table 1 indicate that modulation of CD80 expression by GM-CSF and TNF was not very substantial. But considering that LCs comprise ~1% of total EC population, and only a subset of these LCs might express CD80, the results seem interesting. Also of some import is the fact that trypinization of epidermis to obtain cell suspension (indirect immunofluoresence staining) impairs the expression of CD80 (63b). To avoid this in the future, enzymes such as dispase can be used as a substitute.

Summary

The outcome of the various experiments undertaken in this study has revealed important information about the aspect of LC migration via the engagement of the costimulatory molecule CD80 that is expressed on its surface during maturation. The results sindicate

significant involvement of cytokine TNFα in anti-CD80 induced migration of LC from epidermis. It also showed that cytokine GM-CSF, adhesion molecules ICAM-1 and to lesser extent LFA-1 may possibly be involved in the anti-CD80 induced migration process while ruling out the involvement of IL-1. ELISAs for GM-CSF and TNFα did not detect significant differences in the levels of these cytokines secreted among various groups. These results support the possibilities that cytokine induction is probably not the cause of the anti-CD80 induced effect. The mechanism by which anti-CD80 antibody causes depletion is unknown, though the possibility that these cytokines might exert an affect pror to anti-CD80 induced migratory pathway cannot be ruled out completely.

Table I

Percentage of CD80-positive epidermal cells as obtained by Indirect Immunofluorescence Staining. The epidermal cells were cultured with TNF and GM-CSF for 24 hours, stained with FITC-CD80 Abs and observed under fluorescence microscope

Treatments Percentage of FITC positive cells =>	Control	TNFα	GM-CSF	TNFα + GM-CSF
Trial 1	0.79	3.25	2.5	2.88
Trial 2	0.83	2.1	1.8	3.3

CHAPTER IV

DISCUSSION

There is no doubt that the induction of LC migration is one of the important physiological events occurring in the skin with an imminent effect on the activation state of naive and quiescent T-cells and hence by extension on all T cell dependent cutaneous immune responses (84). Antigen loaded LCs move down the afferent lymphatic vessels to T-cell rich paracortical regions of the lymph node where they encounter and activate T-cells. The activated T cells then enter or "home" towards the original site of inflammation and bring about a secondary phase of the immune response (152). An impressive quantity of data regarding the factors eliciting LC departure from epidermis has accumulated over last few years. The complete understanding of the molecular mechanisms involved in the migration process is still being unraveled. Hence, the knowledge gained so far is mostly fragmentary, and some results are seemingly inconsistent and conflicting. It is of utmost importance to obtain an overall picture to exploit the potential of LCs and by extension DCs, in immunotherapy.

As reported earlier, diverse factors such as antigens, chemical allergens, UV light, FITC, cytokines and several members of chemokine family induce the vectorial movement of LCs (40, 9, 77, 95, 76, 236). The morphological changes influence the functional aspects of LC and involve upregulation of CD80, CD86, CD40, CD54 and MHC II molecules (5). It is imperative to stress that maturation and migration processes occur parallel to each other. Hence, the questions arises whether molecules such as CD80 that are traditionally associated with the maturation process of LC and the

costimulation of virgin and resting T cells, are also involved in the migration of LCs. Though traditionally CD80 and CD86 were associated with regulation of T cell activation versus tolerance, we have hypothesized that CD80 may influence LC egress and localization by triggering signaling pathways on ligation with CD28/CTLA-4 *in vivo*. Thus the crosslinking of the ligand pairs may operate in reciprocal directions. We assumed for our present study that crosslinking of CD80 with its antibody mimics the engagement of CD80 receptor with its ligand CD28 or CTLA-4 on T cells.

In the present endeavor, we have attempted to uncover an unknown facet of CD80. In the course of this, we have investigated and analyzed a triad of questions. Do antibodies against CD80 induce a change in the frequency of LC number in the murine epidermis? If yes then, is the change in the frequency due to migration? If yes again, then do the cytokines influence the anti-CD80 induced migration?

As reported earlier, adhesion molecules and their ligands actively participate in cutaneous inflammatory episodes by directing leukocyte trafficking and by mediating antigen presentation. Modifications in adhesion molecule expression were reflected by changes in the cell morphology including cell depolarization, the appearance of filopods and loss of adherence (13). Anti-ICAM-1 and anti-LFA-1 antibodies have been shown to prevent allo-graft rejections and contact hypersensitivity among others. In particular, ICAM-1 specific antibody has been observed to have had a pronounced effect on contact sensitivity induced LC migration while LFA-1 effected LC migration to a lesser degree (129). Our findings that i.p. administration of antibodies against CD80 causes depletion in LC density can be supported by the fact that anti-ICAM-1 antibody and to a certain extent anti- LFA-1 antibody block the anti-CD80 induced effect. This also allows us to speculate that the depletion may be due to migration of the LC from epidermis.

Therefore, our data agree with the above observations to a certain extent. However, the synergistic effect of anti-ICAM-1 and anti-LFA-1 reported by Ma *et al.* was not seen in our case (129). Our results indicate that both these adhesion molecules must be use a similar mechanism in the induction of migration. The lower effect of anti-LFA-1 might be attributed to a lower level expression of LFA-1 by immunocompetent cell in the epidermis or to the amounts of antibody used as well as the fact that LFA-1 can couple with its ligand ICAM-1 only upon activation. In fact, Ma and others barely detected the presence of LFA-1 on LCs by immunohistochemical techniques and also observed that endothelial cells do not express LFA-1 (129). Activation of LFA-1 on T cells was shown to have occurred via engagement of different cell surface receptors. For instance antibody and CD28 crosslinking (187) and, TCR and MHC II interactions were observed to have had an influence upon the LFA-1 and ICAM-1 interactions (221). Therefore, one possible explanation for blockade of anti-CD80 induced LC migration by anti- ICAM-1 and anti-LFA-1 is that crosslinking of antibody with CD80 initiates LFA-1 and ICAM-1 interaction by producing some unknown signal.

Our next goal was to examine the effect of cytokines that were reported in the literature to have had an effect on LC migration- GM-CSF (89, 168), IL-1α (185), IL-1β , TNFα (41) on anti-CD80 induced effect. Consequently, we examined the effect of anti-cytokine antibodies on anti-CD80 induced LC migration. We found that both anti-TNFα and anti-GM-CSF though the latter to a lesser extent, seemed to have prevented the anti-CD80 induced effect on LC migration. While IL-1α and IL-1β were shown to have had a negligible effect.

Two possibilities that might explain the influence of TNFα and GM-CSF are that the antibody/CD80 crosslinking induces expression of these cytokines and hence the

effect, or that the cytokines were produced upstream of the anti-CD80 induced effect as result of the trauma to tissue which was caused while recovering skin explants. By performing TNFα and GM-CSF ELISAs we tested these possibilities. Both the cytokines were found to be secreted by all the treatment groups in fairly high amounts and no significant difference was established between the control groups and the various groups treated with CD80 specific antibodies. Hence one possible explanation might be that the cytokines TNFα and GM-CSF induce expression of CD80 molecules on LCs *in vitro* culture since their expression on freshly isolated epidermal LCs was very poor (92, 34). Recent evidence also indicates that a dramatic increase in the expression of CD80 occurred on acute myeloid leukemia cells on addition of GM-CSF and other cytokines. Hence, our model contends that the upregulation of CD80 by itself does not cause egress of LC from epidermis but once CD80 binds with antibodies, signals are generated in the form of cytokines (Fig. 7) or chemokines that are eventually responsible for migration of LC.

Some recent works in the literature indicate that IL-1β or TNFα is not sufficient to induce CD80 expression on LCs *in vivo* (149) while others like Salgado showed that TNFα is required for upregulation of CD80 *in vitro* (170). It was also reported by Ozawa *et al.* that while none of the antibodies to IL-1 β, TNFα, or GM-CSF changed the upregulation of Ia antigen, ICAM-1, or CD40 on cultured Langerhans cells, anti-GM-CSF suppressed that of CD80 and CD86. Regardless, taken together the studies indicate that IL-1β is required for the upregulation of Ia, ICAM-1, CD86, and CD40 and not for the upregulation of CD80, while GM-CSF and TNFα are required for the upregulation of CD80 (34, 170, 149, 150).

Our results that anti-TNFα and anti-GM-CSF antibodies induced blockade of anti-CD80 induced LC depletion can be explained based on the above-published works and by evoking the overly-simplistic paradigm we put forward. The findings of Larsen *et al.* also corroborate these observations and link GM-CSF induced upregulation of CD80 with functional maturation and migration of LCs out of skin (116, 177). Our data was found to be statistically significant for anti-TNFα treatment and not for anti-GM-CSF. This inconsistency can be attributed to the observation that GM-CSF apart from its effect on migration also plays a role in LC precursor recruitment from circulating blood to the skin (89). This also explains the partial decrease in LC number on administration of anti-GM-CSF alone.

Alternatively, the effect of TNFα on LC migration can be attributed to its influence on the regulation of the expression of adhesion molecules on LC (96). TNFα induced endothelial E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (V-CAM-1) in all groups, and adhesion molecule ICAM-1 and V-CAM-1 expression by interstitial dermal dendritic cells and ICAM-1 by keratinocytes was observed (74). Interwoven with this is one possiblity that crosslinking of CD80 and its antibodies effectively signals upregulation of ICAM-1 and LFA-1 indirectly and hence the explanation that they mediate anti-CD80 induced migration becomes much more plausible. Another explanation might be that the antibody against CD80 induces chemokine production that directs mobilization of LCs from epidermis. Richins *et al* have shown that antibody against the chemokine MCP-1 prevented depletion induced by anti-CD86 (Richins unpublished).

The exact roles played by cytokines TNFα and GM-CSF or by the adhesion molecules ICAM-1 and LFA-1 in anti-CD80 orchestrated LC migration have still to be

elucidated. Also of the interest is the nature of the signals that regulate expression of the adhesion molecules. Nevertheless, it may be that cytokines like TNFα or chemokines like MCP-1 serve as important mediators of anti-CD80 induced migration.

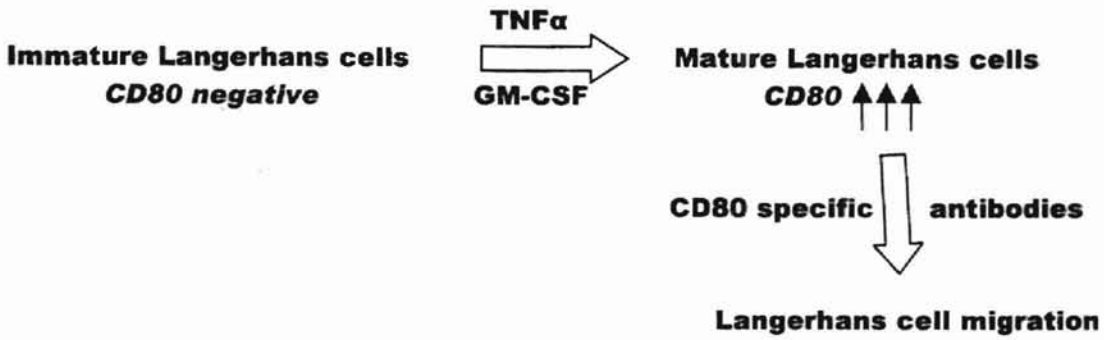

Figure 7. Schematic representation of the mechanism involved in Langerhans cell migration from murine epidermis on treatment with antibodies against CD80 co-stimulatory molecule.

REFERENCES

1. **Aberer, W., G. Stingl, L. A. Stingl-Gazze, and K. Wolff.** 1982. Langerhans cells as stimulator cells in the murine primary epidermal cell-lymphocyte reaction: alteration by UV-B irradiation. J Invest Dermatol. **79**(2):129-35.

2. **Acevedo, A., M. A. del Pozo, A. G. Arroyo, P. Sanchez-Mateos, R. Gonzalez-Amaro, and F. Sanchez-Madrid.** 1993. Distribution of ICAM-3-bearing cells in normal human tissues. Expression of a novel counter-receptor for LFA-1 in epidermal Langerhans cells. Am J Pathol. **143**(3):774-83.

3. **Adams, D. H., and A. R. Lloyd.** 1997. Chemokines: leucocyte recruitment and activation cytokines. Lancet. **349**(9050):490-5.

4. **Aiba, S., and S. I. Katz.** 1990. Phenotypic and functional characteristics of *in vivo*-activated Langerhans cells. J Immunol. **145**(9):2791-6.

5. **Aiba, S., A. Terunuma, H. Manome, and H. Tagami.** 1997. Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. Eur J Immunol. **27**(11):3031-8.

6. **Allison, J. P.** 1994. CD28-B7 interactions in T-cell activation. Curr Opin Immunol. **6**(3):414-9.

7. **Antonopoulos, C., M. Cumberbatch, R. J. Dearman, R. J. Daniel, I. Kimber, and R. W. Groves.** 2001. Functional caspase-1 is required for Langerhans cell migration and optimal contact sensitization in mice. J Immunol. **166**(6):3672-7.

8. **Arai, K. I., F. Lee, A. Miyajima, S. Miyatake, N. Arai, and T. Yokota.** 1990. Cytokines: coordinators of immune and inflammatory responses. Annu Rev Biochem. **59**:783-836.

9. **Asbell, P. A., and T. Kamenar.** 1987. The response of Langerhans cells in the cornea to herpetic keratitis. Curr Eye Res. **6**(1):179-82.

10. **Aubock, J., N. Romani, G. Grubauer, and P. Fritsch.** 1986. HLA-DR expression on keratinocytes is a common feature of diseased skin. Br J Dermatol. **114**(4):465-72.

11. **Baggiolini, M.** 1993. Chemotactic and inflammatory cytokines--CXC and CC proteins. Adv Exp Med Biol. **351**:1-11.

12. **Baggiolini, M., and C. A. Dahinden.** 1994. CC chemokines in allergic inflammation. Immunol Today. **15**(3):127-33.

13. **Ban, E., L. Dupre, E. Hermann, W. Rohn, C. Vendeville, B. Quatannens, P. Ricciardi-Castagnoli, A. Capron, and G. Riveau.** 2000. CpG motifs induce Langerhans cell migration *in vivo*. Int Immunol. **12**(6):737-45.

14.**Berg, E. L., T. Yoshino, L. S. Rott, M. K. Robinson, R. A. Warnock, T. K. Kishimoto, L. J. Picker, and E. C. Butcher.** 1991. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. J Exp Med. **174**(6):1461-6.

15.**Bergstresser, P. R., C. R. Fletcher, and J. W. Streilein.** 1980. Surface densities of Langerhans cells in relation to rodent epidermal sites with special immunologic properties. J Invest Dermatol. **74**(2):77-80.

16.**Bergstresser, P. R., G. B. Toews, and J. W. Streilein.** 1980. Natural and perturbed distributions of Langerhans cells: responses to ultraviolet light, heterotopic skin grafting, and dinitrofluorobenzene sensitization. J Invest Dermatol. **75**(1):73-7.

17.**Bieber, T., H. de la Salle, A. Wollenberg, J. Hakimi, R. Chizzonite, J. Ring, D. Hanau, and C. de la Salle.** 1992. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI). J Exp Med. **175**(5):1285-90.

18.**Birbeck MS, B. A., Everall JD.** 1961. an electron microscopic study of basal melanocytes and high level clear cells (langerhans cells) in vitiligo. J invest dermat. **37**:51.

19.**Blauvelt, A., H. Asada, V. Klaus-Kovtun, D. J. Altman, D. R. Lucey, and S. I. Katz.** 1996. Interleukin-15 mRNA is expressed by human keratinocytes Langerhans cells, and blood-derived dendritic cells and is downregulated by ultraviolet B radiation. J Invest Dermatol. **106**(5):1047-52.

20.**Boise, L. H., A. J. Minn, P. J. Noel, C. H. June, M. A. Accavitti, T. Lindsten, and C. B. Thompson.** 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. Immunity. **3**(1):87-98.

21.**Boorsma, D. M., J. Flier, E. N. van den Brink, S. Sampat, H. L. Walg, R. Willemze, C. P. Tensen, and T. J. Stoof.** 1999. IP-10 mRNA expression in cultured keratinocytes is suppressed by inhibition of protein kinase-C and tyrosine kinase and elevation of cAMP. Cytokine. **11**(7):469-75.

22.**Borkowski, T. A., J. J. Letterio, A. G. Farr, and M. C. Udey.** 1996. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. J Exp Med. **184**(6):2417-22.

23.**Borkowski, T. A., B. J. Van Dyke, K. Schwarzenberger, V. W. McFarland, A. G. Farr, and M. C. Udey.** 1994. Expression of E-cadherin by murine dendritic cells: E-cadherin as a dendritic cell differentiation antigen characteristic of epidermal Langerhans cells and related cells. Eur J Immunol. **24**(11):2767-74.

24.**Bos, J. D., I. Zonneveld, P. K. Das, S. R. Krieg, C. M. van der Loos, and M. L. Kapsenberg.** 1987. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. J Invest Dermatol. **88**(5):569-73.

25.**Bos JD, D. P., Kapsenberg KL.** 1997. The Skin Immune System (SIS), p. 9-16. *In* B. JD (ed.), Sin Immune System: Cutaneous Immunology and Clinical Immunodermatology, 2 ed. CRC Press, Boca Raton.

26.**Breathnach, A. S., W. K. Silvers, J. Smith, and S. Heyner.** 1968. Langerhans cells in mouse skin experimentally deprived of its neural crest component. J Invest Dermatol. **50**(2):147-60.

27.**Bruynzeel-Koomen, C., E. M. van der Donk, P. L. Bruynzeel, M. Capron, G. C. de Gast, and G. C. Mudde.** 1988. Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. Clin Exp Immunol. **74**(1):137-42.

28.**Bruynzeel-Koomen, C., D. F. van Wichen, J. Toonstra, L. Berrens, and P. L. Bruynzeel.** 1986. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. Arch Dermatol Res. **278**(3):199-205.

29.**Burnham, K., L. Robb, C. L. Scott, M. O'Keeffe, and K. Shortman.** 2000. Effect of granulocyte-macrophage colony-stimulating factor on the generation of epidermal Langerhans cells. J Interferon Cytokine Res. 20(12):1071-6.

29a.**Buus, S.** 1994. MHC restricted antigen presentation and T cell recognition. Dan Med Bull. **41**(3):345-58.

30.**Caux, C., C. Dezutter-Dambuyant, D. Schmitt, and J. Banchereau.** 1992. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature. **360**(6401):258-61.

31.**Caux, C., B. Vanbervliet, C. Massacrier, C. Dezutter-Dambuyant, B. de Saint-Vis, C. Jacquet, K. Yoneda, S. Imamura, D. Schmitt, and J. Banchereau.** 1996. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. J Exp Med. **184**(2):695-706.

32.**Cella, M., A. Engering, V. Pinet, J. Pieters, and A. Lanzavecchia.** 1997. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature. **388**(6644):782-7.

33.**Chambers, C. A., D. Cado, T. Truong, and J. P. Allison.** 1997. Thymocyte development is normal in CTLA-4-deficient mice. Proc Natl Acad Sci U S A. **94**(17):9296-301.

34.**Chang, C. H., M. Furue, and K. Tamaki.** 1995. B7-1 expression of Langerhans cells is up-regulated by proinflammatory cytokines, and is down-regulated by interferon-gamma or by interleukin-10. Eur J Immunol. **25**(2):394-8.

35.**Chatila, T., and R. S. Geha.** 1993. Signal transduction by microbial superantigens via MHC class II molecules. Immunol Rev. **131**:43-59.

36.**Chen, H., and R. L. Hendricks.** 1998. B7 costimulatory requirements of T cells at an inflammatory site. J Immunol. **160**(10):5045-52.

37.**Christophers, E.** 1971. Cellular architecture of the stratum corneum. J Invest Dermatol. **56**(3):165-9.

38.**Cosimi, A. B., D. Conti, F. L. Delmonico, F. I. Preffer, S. L. Wee, R. Rothlein, R. Faanes, and R. B. Colvin.** 1990. *In vivo* effects of monoclonal antibody to ICAM-1 (CD54) in nonhuman primates with renal allografts. J Immunol. **144**(12):4604-12.

39.**Cumberbatch, M., R. J. Dearman, C. Antonopoulos, R. W. Groves, and I. Kimber.** 2001. Interleukin (IL)-18 induces Langerhans cell migration by a tumour necrosis factor-alpha- and IL-1beta-dependent mechanism. Immunology. **102**(3):323-30.

40.**Cumberbatch, M., R. J. Dearman, C. E. Griffiths, and I. Kimber.** 2000. Langerhans cell migration. Clin Exp Dermatol. **25**(5):413-8.

41.**Cumberbatch, M., R. J. Dearman, and I. Kimber.** 1997. Interleukin 1 beta and the stimulation of Langerhans cell migration: comparisons with tumour necrosis factor alpha. Arch Dermatol Res. **289**(5):277-84.

42.**Cumberbatch, M., I. Fielding, and I. Kimber.** 1994. Modulation of epidermal Langerhans' cell frequency by tumour necrosis factor-alpha. Immunology. **81**(3):395-401.

43.**Cumberbatch, M., and I. Kimber.** 1992. Dermal tumour necrosis factor-alpha induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. Immunology. **75**(2):257-63.

44.**Cumberbatch, M., S. W. Peters, S. J. Gould, and I. Kimber.** 1992. Intercellular adhesion molecule-1 (ICAM-1) expression by lymph node dendritic cells: comparison with epidermal Langerhans cells. Immunol Lett. **32**(2):105-10.

45.**Dandie, G. W., G. J. Clydesdale, I. Jacobs, and H. K. Muller.** 1998. Effects of UV on the migration and function of epidermal antigen presenting cells. Mutat Res. **422**(1):147-54.

46.**Danis, V. A., M. Millington, V. J. Hyland, and D. Grennan.** 1995. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol. **99**(2):303-10.

47.**De Creus, A., K. Van Beneden, T. Taghon, F. Stolz, V. Debacker, J. Plum, and G. Leclercq.** 2000. Langerhans cells that have matured *in vivo* in the absence of T cells are fully capable of inducing a helper CD4 as well as a cytotoxic CD8 response. J Immunol. **165**(2):645-53.

48.**De Fazio, S. R., and J. J. Gozzo.** 2000. Role of graft interleukin-10 expression in the tolerogenicity of neonatal skin allografts. Transplantation. **70**(9):1371-7.

49.**Dieu-Nosjean, M. C., C. Massacrier, B. Homey, B. Vanbervliet, J. J. Pin, A. Vicari, S. Lebecque, C. Dezutter-Dambuyant, D. Schmitt, A. Zlotnik, and C. Caux.** 2000. Macrophage inflammatory protein 3alpha is expressed at inflamed epithelial surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. J Exp Med. **192**(5):705-18.

50.**Dinarello, C. A.** 1991. Interleukin-1 and interleukin-1 antagonism. Blood. **77**(8):1627-52.

51.**Dinarello, C. A.** 1989. Interleukin-1 and its biologically related cytokines. Adv Immunol. **44**:153-205.

52.**Dinarello, C. A., J. G. Cannon, S. M. Wolff, H. A. Bernheim, B. Beutler, A. Cerami, I. S. Figari, M. A. Palladino, Jr., and J. V. O'Connor.** 1986. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J Exp Med. **163**(6):1433-50.

53.**Dower, S. K., S. R. Kronheim, T. P. Hopp, M. Cantrell, M. Deeley, S. Gillis, C. S. Henney, and D. L. Urdal.** 1986. The cell surface receptors for interleukin-1 alpha and interleukin-1 beta are identical. Nature. **324**(6094):266-8.

54.**Dustin, M. L., K. H. Singer, D. T. Tuck, and T. A. Springer.** 1988. Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule 1 (ICAM-1). J Exp Med. **167**(4):1323-40.

55.**Dustin, M. L., and T. A. Springer.** 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature. **341**(6243):619-24.

56.**Elias, P. M., L. C. Wood, and K. R. Feingold.** 1999. Epidermal pathogenesis of inflammatory dermatoses. Am J Contact Dermat. **10**(3):119-26.

57.**Enk, A. H., V. L. Angeloni, M. C. Udey, and S. I. Katz.** 1993. An essential role for Langerhans cell-derived IL-1 beta in the initiation of primary immune responses in skin. J Immunol. **150**(9):3698-704.

58.**Enk, A. H., and S. I. Katz.** 1995. Contact sensitivity as a model for T-cell activation in skin. J Invest Dermatol. **105**(1 Suppl):80S-83S.

59.**Enk, A. H., and S. I. Katz.** 1992. Early molecular events in the induction phase of contact sensitivity. Proc Natl Acad Sci U S A. **89**(4):1398-402.

60.**Fanslow, W. C., K. N. Clifford, L. S. Park, A. S. Rubin, R. F. Voice, M. P. Beckmann, and M. B. Widmer.** 1991. Regulation of alloreactivity *in vivo* by IL-4 and the soluble IL-4 receptor. J Immunol. **147**(2):535-40.

61.**Faustman, D. L., R. M. Steinman, H. M. Gebel, V. Hauptfeld, J. M. Davie, and P. E. Lacy.** 1984. Prevention of rejection of murine islet allografts by pretreatment with anti-dendritic cell antibody. Proc Natl Acad Sci U S A. **81**(12):3864-8.

62.**Fenton, M. J., M. W. Vermeulen, B. D. Clark, A. C. Webb, and P. E. Auron.** 1988. Human pro-IL-1 beta gene expression in monocytic cells is regulated by two distinct pathways. J Immunol. **140**(7):2267-73.

63a.**Fichtelius, K. E., O. Groth, and S. Liden.** 1970. The skin, a first level lymphoid organ? Int Arch Allergy Appl Immunol. **37**(6):607-20.

63b.**Fleming, T. E., W. S. Mirando, U. Trefzer, K. A. Tubesing, and C. A. Elmets.** 1993. In situ expression of a B7-like adhesion molecule on keratinocytes from human epidermis. J Invest Dermatol. 101(5):754-8.

64.**Freeman, G. J., V. A. Boussiotis, A. Anumanthan, G. M. Bernstein, X. Y. Ke, P. D. Rennert, G. S. Gray, J. G. Gribben, and L. M. Nadler.** 1995. B7-1 and B7-2 do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. Immunity. **2**(5):523-32.

65.**Frelinger, J. G., L. Hood, S. Hill, and J. A. Frelinger.** 1979. Mouse epidermal Ia molecules have a bone marrow origin. Nature. **282**(5736):321-3.

66.**Gabrilovich, D. I., G. M. Woods, S. Patterson, J. J. Harvey, and S. C. Knight.** 1994. Retrovirus-induced immunosuppression via blocking of dendritic cell migration and down-regulation of adhesion molecules. Immunology. **82**(1):82-7.

67.**Geiger, E., R. Magerstaedt, J. H. Wessendorf, S. Kraft, D. Hanau, and T. Bieber.** 2000. IL-4 induces the intracellular expression of the alpha chain of the high-affinity receptor for IgE in *in vitro*-generated dendritic cells. J Allergy Clin Immunol. **105**(1 Pt 1):150-6.

68.**Geissmann, F., P. Revy, A. Regnault, Y. Lepelletier, M. Dy, N. Brousse, S. Amigorena, O. Hermine, and A. Durandy.** 1999. TGF-beta 1 prevents the noncognate maturation of human dendritic Langerhans cells. J Immunol. **162**(8):4567-75.

69.**Georgopoulos, K., M. Bigby, J. H. Wang, A. Molnar, P. Wu, S. Winandy, and A. Sharpe.** 1994. The Ikaros gene is required for the development of all lymphoid lineages. Cell. **79**(1):143-56.

70.**Girolomoni, G., G. Zambruno, R. Manfredini, V. Zacchi, S. Ferrari, A. Cossarizza, and A. Giannetti.** 1994. Expression of B7 costimulatory molecule in cultured human epidermal Langerhans cells is regulated at the mRNA level. J Invest Dermatol. **103**(1):54-9.

71.**Godiska, R., D. Chantry, C. J. Raport, S. Sozzani, P. Allavena, D. Leviten, A. Mantovani, and P. W. Gray.** 1997. Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J Exp Med. **185**(9):1595-604.

72.**Grandaliano, G., A. J. Valente, M. M. Rozek, and H. E. Abboud.** 1994. Gamma interferon stimulates monocyte chemotactic protein (MCP-1) in human mesangial cells. J Lab Clin Med. **123**(2):282-9.

73.**Gribben, J. G., G. J. Freeman, V. A. Boussiotis, P. Rennert, C. L. Jellis, E. Greenfield, M. Barber, V. A. Restivo, Jr., X. Ke, G. S. Gray, and et al.** 1995. CTLA4 mediates antigen-specific apoptosis of human T cells. Proc Natl Acad Sci U S A. **92**(3):811-5.

74.**Groves, R. W., M. H. Allen, E. L. Ross, J. N. Barker, and D. M. MacDonald.** 1995. Tumour necrosis factor alpha is pro-inflammatory in normal human skin and modulates cutaneous adhesion molecule expression. Br J Dermatol. **132**(3):345-52.

75.**Gueniche, A., J. Viac, M. Charveron, and D. Schmitt.** 1994. Effect of gamma-interferon on IL-1 alpha, beta and receptor antagonist production by normal human keratinocytes. Exp Dermatol. **3**(3):113-8.

76.**Halliday, G. M., and A. D. Lucas.** 1993. Protein kinase C transduces the signal for Langerhans' cell migration from the epidermis. Immunology. **79**(4):621-6.

77.**Halliday, G. M., V. E. Reeve, and R. S. Barnetson.** 1991. Langerhans cell migration into ultraviolet light-induced squamous skin tumors is unrelated to anti-tumor immunity. J Invest Dermatol. **97**(5):830-4.

78.**Hammar, S.** 1988. Langerhans cells. Pathol Annu. **23**(Pt 2):293-328.

79.**Hanau, D., M. Fabre, D. A. Schmitt, J. L. Stampf, J. C. Garaud, T. Bieber, E. Grosshans, C. Benezra, and J. P. Cazenave.** 1987. Human epidermal Langerhans cells internalize by receptor-mediated endocytosis T6 (CD1 "NA1/34") surface antigen. Birbeck granules are involved in the intracellular traffic of the T6 antigen. J Invest Dermatol. **89**(2):172-7.

80.**Heagy, W., C. Walterbangh, and E. Martz.** 1984. Potent ability of anti-LFA-1 monoclonal antibody to prolong allograft survival. Transplantation. **37**(5):520-3.

81.**Heufler, C., F. Koch, and G. Schuler.** 1988. Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. J Exp Med. **167**(2):700-5.

82.**Hurme, M., and E. Serkkola.** 1991. Different activation signals are required for the expression of interleukin-1 alpha and beta genes in human monocytes. Scand J Immunol. **33**(6):713-8.

83.**Inaba, K., M. Inaba, M. Witmer-Pack, K. Hatchcock, R. Hodes, and R. M. Steinman.** 1995. Expression of B7 costimulator molecules on mouse dendritic cells. Adv Exp Med Biol. **378**:65-70.

84.**Inaba, K., G. Schuler, M. D. Witmer, J. Valinksy, B. Atassi, and R. M. Steinman.** 1986. Immunologic properties of purified epidermal Langerhans cells. Distinct requirements for stimulation of unprimed and sensitized T lymphocytes. J Exp Med. **164**(2):605-13.

85.**Isobe, M., H. Yagita, K. Okumura, and A. Ihara.** 1992. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. Science. **255**(5048):1125-7.

86.**Ito, T., M. Inaba, K. Inaba, J. Toki, S. Sogo, T. Iguchi, Y. Adachi, K. Yamaguchi, R. Amakawa, J. Valladeau, S. Saeland, S. Fukuhara, and S. Ikehara.** 1999. A CD1a+/CD11c+ subset of human blood dendritic cells is a direct precursor of Langerhans cells. J Immunol. **163**(3):1409-19.

87.**June, C. H., J. A. Bluestone, L. M. Nadler, and C. B. Thompson.** 1994. The B7 and CD28 receptor families. Immunol Today. **15**(7):321-31.

88.**Kang, K., M. Kubin, K. D. Cooper, S. R. Lessin, G. Trinchieri, and A. H. Rook.** 1996. IL-12 synthesis by human Langerhans cells. J Immunol. **156**(4):1402-7.

89.**Kaplan, G., G. Walsh, L. S. Guido, P. Meyn, R. A. Burkhardt, R. M. Abalos, J. Barker, P. A. Frindt, T. T. Fajardo, R. Celona, and et al.** 1992. Novel responses of human skin to intradermal recombinant granulocyte/macrophage-colony-stimulating factor: Langerhans cell recruitment, keratinocyte growth, and enhanced wound healing. J Exp Med. **175**(6):1717-28.

90.**Katz, S. I., K. Tamaki, and D. H. Sachs.** 1979. Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature. **282**(5736):324-6.

91.**Kaushansky, K., V. C. Broudy, J. M. Harlan, and J. W. Adamson.** 1988. Tumor necrosis factor-alpha and tumor necrosis factor-beta (lymphotoxin) stimulate the production of granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and IL-1 *in vivo*. J Immunol. **141**(10):3410-5.

92.**Kawamura, T., and M. Furue.** 1995. Comparative analysis of B7-1 and B7-2 expression in Langerhans cells: differential regulation by T helper type 1 and T helper type 2 cytokines. Eur J Immunol. **25**(7):1913-7.

93.**Kearney, E. R., T. L. Walunas, R. W. Karr, P. A. Morton, D. Y. Loh, J. A. Bluestone, and M. K. Jenkins.** 1995. Antigen-dependent clonal expansion of a trace population of antigen-specific CD4+ T cells in vivo is dependent on CD28 costimulation and inhibited by CTLA-4. J Immunol. **155**(3):1032-6.

94.**Khoury, S. J., L. Gallon, R. R. Verburg, A. Chandraker, R. Peach, P. S. Linsley, L. A. Turka, W. W. Hancock, and M. H. Sayegh.** 1996. Ex vivo treatment of antigen-presenting cells with CTLA4Ig and encephalitogenic peptide prevents experimental autoimmune encephalomyelitis in the Lewis rat. J Immunol. **157**(8):3700-5.

95.**Kimber, I., and M. Cumberbatch.** 1992. Dendritic cells and cutaneous immune responses to chemical allergens. Toxicol Appl Pharmacol. **117**(2):137-46.

96.**Kimber, I., M. Cumberbatch, R. J. Dearman, M. Bhushan, and C. E. Griffiths.** 2000. Cytokines and chemokines in the initiation and regulation of epidermal Langerhans cell mobilization. Br J Dermatol. **142**(3):401-12.

97.**Kitajima, T., K. Ariizumi, M. Mohamadazadeh, D. Edelbaum, P. R. Bergstresser, and A. Takashima.** 1995. T cell-dependent secretion of IL-1 beta by a dendritic cell line (XS52) derived from murine epidermis. J Immunol. **155**(8):3794-800.

98.**Kitazawa, T., and J. W. Streilein.** 2000. Hapten-specific tolerance promoted by calcitonin gene-related peptide. J Invest Dermatol. **115**(6):942-8.

99.**Klareskog, L., U. Tjernlund, U. Forsum, and P. A. Peterson.** 1977. Epidermal Langerhans cells express Ia antigens. Nature. **268**(5617):248-50.

100.**Knight, S. C., J. Krejci, M. Malkovsky, V. Colizzi, A. Gautam, and G. L. Asherson.** 1985. The role of dendritic cells in the initiation of immune responses to contact sensitizers. I. *In vivo* exposure to antigen. Cell Immunol. **94**(2):427-34.

101.**Koch, F., C. Heufler, E. Kampgen, D. Schneeweiss, G. Bock, and G. Schuler.** 1990. Tumor necrosis factor alpha maintains the viability of murine epidermal Langerhans cells in culture, but in contrast to granulocyte/macrophage colony-stimulating factor, without inducing their functional maturation. J Exp Med. **171**(1):159-71.

102.**Kock, A., T. Schwarz, R. Kirnbauer, A. Urbanski, P. Perry, J. C. Ansel, and T. A. Luger.** 1990. Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med. **172**(6):1609-14.

103.**Kolde, G., and J. Knop.** 1987. Different cellular reaction patterns of epidermal Langerhans cells after application of contact sensitizing, toxic, and tolerogenic compounds. A comparative ultrastructural and morphometric time-course analysis. J Invest Dermatol. **89**(1):19-23.

104.**Kootstra, C. J., D. M. Van Der Giezen, J. H. Van Krieken, E. De Heer, and J. A. Bruijn.** 1997. Effective treatment of experimental lupus nephritis by combined administration of anti-CD11a and anti-CD54 antibodies. Clin Exp Immunol. **108**(2):324-32.

105.**Kripke, M. L., and M. S. Fisher.** 1976. Immunologic parameters of ultraviolet carcinogenesis. J Natl Cancer Inst. **57**(1):211-5.

106.**Kripke, M. L., C. G. Munn, A. Jeevan, J. M. Tang, and C. Bucana.** 1990. Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. J Immunol. **145**(9):2833-8.

107.**Krummel, M. F., and J. P. Allison.** 1995. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. **182**(2):459-65.

108.**Kuchroo, V. K., M. P. Das, J. A. Brown, A. M. Ranger, S. S. Zamvil, R. A. Sobel, H. L. Weiner, N. Nabavi, and L. H. Glimcher.** 1995. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell. **80**(5):707-18.

109.**Kuhlman, P., V. T. Moy, B. A. Lollo, and A. A. Brian.** 1991. The accessory function of murine intercellular adhesion molecule-1 in T lymphocyte activation. Contributions of adhesion and co-activation. J Immunol. **146**(6):1773-82.

110.**Kutsch, C. L., D. A. Norris, and W. P. Arend.** 1993. Tumor necrosis factor-alpha induces interleukin-1 alpha and interleukin-1 receptor antagonist production by cultured human keratinocytes. J Invest Dermatol. **101**(1):79-85.

111.**Lang, R. A., D. Metcalf, R. A. Cuthbertson, I. Lyons, E. Stanley, A. Kelso, G. Kannourakis, D. J. Williamson, G. K. Klintworth, T. J. Gonda, and et al.** 1987. Transgenic mice expressing a hemopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. Cell. **51**(4):675-86.

112.**Langerhans, P.** 1868. Uber die nerven der menschlichen haut. Virchows Arch [Pathol Anat]. **44**:325-337.

113.**Lanzavecchia, A.** 1990. Receptor-mediated antigen uptake and its effect on antigen presentation to class II-restricted T lymphocytes. Annu Rev Immunol. **8**:773-93.

114.**Lappe, M.** 1997. The Tao of Immunology: a revolutionary new understanding of our body's defenses. Plenum Press.

115.**Larregina, A., A. Morelli, E. Kolkowski, and L. Fainboim.** 1996. Flow cytometric analysis of cytokine receptors on human Langerhans' cells. Changes observed after short-term culture. Immunology. **87**(2):317-25.

116.**Larsen, C. P., S. C. Ritchie, R. Hendrix, P. S. Linsley, K. S. Hathcock, R. J. Hodes, R. P. Lowry, and T. C. Pearson.** 1994. Regulation of immunostimulatory function and costimulatory molecule (B7-1 and B7-2) expression on murine dendritic cells. J Immunol. **152**(11):5208-19.

117.**Larsen, C. P., R. M. Steinman, M. Witmer-Pack, D. F. Hankins, P. J. Morris, and J. M. Austyn.** 1990. Migration and maturation of Langerhans cells in skin transplants and explants. J Exp Med. **172**(5):1483-93.

118.**Larson, R. S., and T. A. Springer.** 1990. Structure and function of leukocyte integrins. Immunol Rev. **114**:181-217.

119.**Leibl, H., J. Hutterer, H. Korschan, G. Schuler, M. Tani, E. Tschachler, N. Romani, K. Wolff, and G. Stingl.** 1985. Expression of the Ly-5 alloantigenic system on epidermal cells. J Invest Dermatol. **84**(2):91-5.

120.**Lenschow, D. J., Y. Zeng, K. S. Hathcock, L. A. Zuckerman, G. Freeman, J. R. Thistlethwaite, G. S. Gray, R. J. Hodes, and J. A. Bluestone.** 1995. Inhibition of transplant rejection following treatment with anti-B7-2 and anti-B7-1 antibodies. Transplantation. **60**(10):1171-8.

121.**Lessard, R. J., K. Wolff, and R. K. Winkelmann.** 1968. The disappearance and regeneration of Langerhans cells following epidermal injury. J Invest Dermatol. **50**(2):171-9.

122.**Linsley, P. S., E. A. Clark, and J. A. Ledbetter.** 1990. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc Natl Acad Sci U S A. **87**(13):5031-5.

123.**Linsley, P. S., P. M. Wallace, J. Johnson, M. G. Gibson, J. L. Greene, J. A. Ledbetter, C. Singh, and M. A. Tepper.** 1992. Immunosuppression *in vivo* by a soluble form of the CTLA-4 T cell activation molecule. Science. **257**(5071):792-5.

124.**Lu, L., S. Qian, P. A. Hershberger, W. A. Rudert, D. H. Lynch, and A. W. Thomson.** 1997. Fas ligand (CD95L) and B7 expression on dendritic cells provide counter-regulatory signals for T cell survival and proliferation. J Immunol. **158**(12):5676-84.

125.**Ludewig, B., J. Holzmeister, M. Gentile, H. R. Gelderblom, K. Rokos, Y. Becker, and G. Pauli.** 1995. Replication pattern of human immunodeficiency virus type 1 in mature Langerhans cells. J Gen Virol. **76**(Pt 6):1317-25.

126.**Luger, T. A., D. Kalden, T. E. Scholzen, and T. Brzoska.** 1999. alpha-melanocyte-stimulating hormone as a mediator of tolerance induction. Pathobiology. **67**(5-6):318-21.

127.**Luger, T. A., and T. Schwarz.** 1990. Evidence for an epidermal cytokine network. J Invest Dermatol. **95**(6 Suppl):100S-104S.

128.**Luger, T. A., B. M. Stadler, S. I. Katz, and J. J. Oppenheim.** 1981. Epidermal cell (keratinocyte)-derived thymocyte-activating factor (ETAF). J Immunol. **127**(4):1493-8.

129.**Ma, J., J. H. Wang, Y. J. Guo, M. S. Sy, and M. Bigby.** 1994. *In vivo* treatment with anti-ICAM-1 and anti-LFA-1 antibodies inhibits contact sensitization-induced migration of epidermal Langerhans cells to regional lymph nodes. Cell Immunol. **158**(2):389-99.

130.**Macatonia, S. E., S. C. Knight, A. J. Edwards, S. Griffiths, and P. Fryer.** 1987. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. J Exp Med. **166**(6):1654-67.

131.**Mackay, C. R., and B. A. Imhof.** 1993. Cell adhesion in the immune system. Immunol Today. **14**(3):99-102.

132.**Manz, M. G., D. Traver, T. Miyamoto, I. L. Weissman, and K. Akashi.** 2001. Dendritic cell potentials of early lymphoid and myeloid progenitors. Blood. **97**(11):3333-41.

133.**Masson, P.** 1926. Les Naevi pigmentaires, tumeurs nerveuses. Ann Anat path. **3**:417-657.

134.**Matsue, H., and A. Takashima.** 1999. Apoptosis in dendritic cell biology. J Dermatol Sci. **20**(3):159-71.

135.**McLellan, A. D., G. C. Starling, L. A. Williams, B. D. Hock, and D. N. Hart.** 1995. Activation of human peripheral blood dendritic cells induces the CD86 co-stimulatory molecule. Eur J Immunol. **25**(7):2064-8.

136.**Merad, M., L. Fong, J. Bogenberger, and E. G. Engleman.** 2000. Differentiation of myeloid dendritic cells into CD8alpha-positive dendritic cells *in vivo*. Blood. **96**(5):1865-72.

137.**Mizumoto, N.** 1999. [Analysis of contact hypersensitivity response in human monocyte chemoattractant protein (MCP)-1 transgenic mice]. Hokkaido Igaku Zasshi. **74**(3):199-216.

138.**Mohamadzadeh, M., M. Muller, T. Hultsch, A. Enk, J. Saloga, and J. Knop.** 1994. Enhanced expression of IL-8 in normal human keratinocytes and human keratinocyte cell line HaCaT *in vitro* after stimulation with contact sensitizers, tolerogens and irritants. Exp Dermatol. **3**(6):298-303.

139.**Morgan, D. A., F. W. Ruscetti, and R. Gallo.** 1976. Selective *in vitro* growth of T lymphocytes from normal human bone marrows. Science. **193**(4257):1007-8.

140.**Murphy, G. F., A. K. Bhan, S. Sato, T. J. Harrist, and M. C. Mihm, Jr.** 1981. Characterization of Langerhans cells by the use of monoclonal antibodies. Lab Invest. **45**(5):465-8.

141.**Nakamura, K., I. R. Williams, and T. S. Kupper.** 1995. Keratinocyte-derived monocyte chemoattractant protein 1 (MCP-1): analysis in a transgenic model demonstrates MCP-1 can recruit dendritic and Langerhans cells to skin. J Invest Dermatol. **105**(5):635-43.

142.**Nakano, Y.** 1998. Antigen-presenting cell function of epidermal cells activated by hapten application. Br J Dermatol. **138**(5):786-94.

143.**Nishio, M., and E. R. Podack.** 1996. Rapid induction of tumor necrosis factor cytotoxicity in naive splenic T cells by simultaneous CD80 (B7.1) and CD54 (ICAM-1) co-stimulation. Eur J Immunol. **26**(9):2160-4.

144.**Nishio, M., J. Spielman, R. K. Lee, D. L. Nelson, and E. R. Podack.** 1996. CD80 (B7.1) and CD54 (intracellular adhesion molecule-1) induce target cell susceptibility to promiscuous cytotoxic T cell lysis. J Immunol. **157**(10):4347-53.

145.**Nordeng, T. W., J. P. Gorvel, and O. Bakke.** 1998. Intracellular transport of molecules engaged in the presentation of exogenous antigens. Curr Top Microbiol Immunol. **232**:179-215.

146.**Nuriya, S., H. Yagita, K. Okumura, and M. Azuma.** 1996. The differential role of CD86 and CD80 co-stimulatory molecules in the induction and the effector phases of contact hypersensitivity. Int Immunol. **8**(6):917-26.

147.**Obochi, M. O., L. G. Ratkay, and J. G. Levy.** 1997. Prolonged skin allograft survival after photodynamic therapy associated with modification of donor skin antigenicity. Transplantation. **63**(6):810-7.

148.**Okamoto, T., T. Matsuyama, S. Mori, Y. Hamamoto, N. Kobayashi, N. Yamamoto, S. F. Josephs, F. Wong-Staal, and K. Shimotohno.** 1989. Augmentation of human immunodeficiency virus type 1 gene expression by tumor necrosis factor alpha. AIDS Res Hum Retroviruses. **5**(2):131-8.

149.**Ozawa, H., S. Aiba, Nakagawa, and H. Tagami.** 1996. Interferon-gamma and interleukin-10 inhibit antigen presentation by Langerhans cells for T helper type 1 cells by suppressing their CD80 (B7-1) expression. Eur J Immunol. **26**(3):648-52.

150.**Ozawa, H., S. Nakagawa, H. Tagami, and S. Aiba.** 1996. Interleukin-1 beta and granulocyte-macrophage colony-stimulating factor mediate Langerhans cell maturation differently. J Invest Dermatol. **106**(3):441-5.

151.**Park, W., C. M. Weyand, D. Schmidt, and J. J. Goronzy.** 1997. Co-stimulatory pathways controlling activation and peripheral tolerance of human CD4+CD28- T cells. Eur J Immunol. **27**(5):1082-90.

152.**Parrott, D. M., and P. C. Wilkinson.** 1981. Lymphocyte locomotion and migration. Prog Allergy. **28**:193-284.

153.**Peguet-Navarro, J., C. Dalbiez-Gauthier, F. M. Rattis, C. Van Kooten, J. Banchereau, and D. Schmitt.** 1995. Functional expression of CD40 antigen on human epidermal Langerhans cells. J Immunol. **155**(9):4241-7.

154.**Picker, L. J., T. K. Kishimoto, C. W. Smith, R. A. Warnock, and E. C. Butcher.** 1991. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature. **349**(6312):796-9.

155.**Pitzalis, C., A. Cauli, N. Pipitone, C. Smith, J. Barker, A. Marchesoni, G. Yanni, and G. S. Panayi.** 1996. Cutaneous lymphocyte antigen-positive T lymphocytes preferentially migrate to the skin but not to the joint in psoriatic arthritis. Arthritis Rheum. **39**(1):137-45.

156.**Price, A. A., M. Cumberbatch, I. Kimber, and A. Ager.** 1997. Alpha 6 integrins are required for Langerhans cell migration from the epidermis. J Exp Med. **186**(10):1725-35.

157.**Prickett, T. C., J. L. McKenzie, and D. N. Hart.** 1992. Adhesion molecules on human tonsil dendritic cells. Transplantation. **53**(2):483-90.

158.**Raines, E. W., S. K. Dower, and R. Ross.** 1989. Interleukin-1 mitogenic activity for fibroblasts and smooth muscle cells is due to PDGF-AA. Science. **243**(4889):393-6.

159.**Rattis, F. M., J. Peguet-Navarro, M. J. Staquet, C. Dezutter-Dambuyant, P. Courtellemont, G. Redziniak, and D. Schmitt.** 1996. Expression and function of B7-1

(CD80) and B7-2 (CD86) on human epidermal Langerhans cells. Eur J Immunol. **26**(2):449-53.

160.**Razi-Wolf, Z., L. D. Falo, Jr., and H. Reiser.** 1994. Expression and function of the costimulatory molecule B7 on murine Langerhans cells: evidence for an alternative CTLA-4 ligand. Eur J Immunol. **24**(4):805-11.

161.**Reis e Sousa, C., P. D. Stahl, and J. M. Austyn.** 1993. Phagocytosis of antigens by Langerhans cells *in vitro*. J Exp Med. **178**(2):509-19.

162.**Reiser, H., and E. E. Schneeberger.** 1996. Expression and function of B7-1 and B7-2 in hapten-induced contact sensitivity. Eur J Immunol. **26**(4):880-5.

163.**Reynolds, N. J., J. Y. Yi, G. J. Fisher, K. D. Cooper, J. J. Voorhees, and C. E. Griffiths.** 1995. Down-regulation of Langerhans cell protein kinase C-beta isoenzyme expression in inflammatory and hyperplastic dermatoses. Br J Dermatol. **133**(2):157-67.

164.**Rieger, A., B. Wang, O. Kilgus, K. Ochiai, D. Mauerer, D. Fodinger, J. P. Kinet, and G. Stingl.** 1992. Fc epsilon RI mediates IgE binding to human epidermal Langerhans cells. J Invest Dermatol. **99**(5):30S-32S.

165.**Romani, N., A. Lenz, H. Glassel, H. Stossel, U. Stanzl, O. Majdic, P. Fritsch, and G. Schuler.** 1989. Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. J Invest Dermatol. **93**(5):600-9.

166.**Romani, N., and G. Schuler.** 1992. The immunologic properties of epidermal Langerhans cells as a part of the dendritic cell system. Springer Semin Immunopathol. **13**(3-4):265-79.

167.**Ruef, C., and D. L. Coleman.** 1990. Granulocyte-macrophage colony-stimulating factor: pleiotropic cytokine with potential clinical usefulness. Rev Infect Dis. **12**(1):41-62.

168.**Rupec, R., R. Magerstaedt, C. G. Schirren, E. Sander, and T. Bieber.** 1996. Granulocyte/macrophage-colony-stimulating factor induces the migration of human epidermal Langerhans cells *in vitro*. Exp Dermatol. **5**(2):115-9.

169.**Saban, M. R., R. Saban, D. Bjorling, and M. Haak-Frendscho.** 1997. Involvement of leukotrienes, TNF-alpha, and the LFA-1/ICAM-1 interaction in substance P-induced granulocyte infiltration. J Leukoc Biol. **61**(4):445-51.

170.**Salgado, C. G., K. Nakamura, M. Sugaya, Y. Tada, A. Asahina, S. Fukuda, Y. Koyama, S. Irie, and K. Tamaki.** 1999. Differential effects of cytokines and immunosuppressive drugs on CD40, B7-1, and B7-2 expression on purified epidermal Langerhans cells1. J Invest Dermatol. **113**(6):1021-7.

171.**Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia.** 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med. **182**(2):389-400.

172.**Sallusto, F., and A. Lanzavecchia.** 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med. **179**(4):1109-18.

173.**Saren, P., H. G. Welgus, and P. T. Kovanen.** 1996. TNF-alpha and IL-1beta selectively induce expression of 92-kDa gelatinase by human macrophages. J Immunol. **157**(9):4159-65.

174.**Sauder, D. N., C. A. Dinarello, and V. B. Morhenn.** 1984. Langerhans cell production of interleukin-1. J Invest Dermatol. **82**(6):605-7.

175.**Scheipers, P., and H. Reiser.** 1998. Role of the CTLA-4 receptor in T cell activation and immunity. Physiologic function of the CTLA-4 receptor. Immunol Res. **18**(2):103-15.

176.**Schopf, R. E., A. Hoffmann, M. Jung, B. Morsches, and K. Bork.** 1986. Stimulation of T cells by autologous mononuclear leukocytes and epidermal cells in psoriasis. Arch Dermatol Res. **279**(2):89-94.

177.**Schreiber, S., O. Kilgus, E. Payer, R. Kutil, A. Elbe, C. Mueller, and G. Stingl.** 1992. Cytokine pattern of Langerhans cells isolated from murine epidermal cell cultures. J Immunol. **149**(11):3524-34.

178.**Schroder, J. M.** 1995. Cytokine networks in the skin. J Invest Dermatol. **105**(1 Suppl):20S-24S.

179.**Schuler, G., and R. M. Steinman.** 1985. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells *in vitro*. J Exp Med. **161**(3):526-46.

180.**Schwartz, R. H., D. L. Mueller, M. K. Jenkins, and H. Quill.** 1989. T-cell clonal anergy. Cold Spring Harb Symp Quant Biol. **54**(Pt 2):605-10.

181.**Schwarz, A., R. Bhardwaj, Y. Aragane, K. Mahnke, H. Riemann, D. Metze, T. A. Luger, and T. Schwarz.** 1995. Ultraviolet-B-induced apoptosis of keratinocytes: evidence for partial involvement of tumor necrosis factor-alpha in the formation of sunburn cells. J Invest Dermatol. **104**(6):922-7.

182.**Schwarz, T., and T. A. Luger.** 1989. Effect of UV irradiation on epidermal cell cytokine production. J Photochem Photobiol B. **4**(1):1-13.

183.**Schwarzenberger, K., and M. C. Udey.** 1996. Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression in situ. J Invest Dermatol. **106**(3):553-8.

184.**Schweitzer, A. N., and A. H. Sharpe.** 1998. Studies using antigen-presenting cells lacking expression of both B7-1 (CD80) and B7-2 (CD86) show distinct requirements for B7 molecules during priming versus restimulation of Th2 but not Th1 cytokine production. J Immunol. **161**(6):2762-71.

185.**Shankar, G., J. Johnson, L. Kuschel, M. Richins, and K. Burnham.** 1999. Protein-kinase-specific inhibitors block Langerhans' cell migration by inhibiting interleukin-1alpha release. Immunology. **96**(2):230-5.

186.**Shimada, S., S. W. Caughman, S. O. Sharrow, D. Stephany, and S. I. Katz.** 1987. Enhanced antigen-presenting capacity of cultured Langerhans' cells is associated with markedly increased expression of Ia antigen. J Immunol. **139**(8):2551-5.

187.**Shimizu, Y., G. A. van Seventer, E. Ennis, W. Newman, K. J. Horgan, and S. Shaw.** 1992. Crosslinking of the T cell-specific accessory molecules CD7 and CD28 modulates T cell adhesion. J Exp Med. **175**(2):577-82.

188.**Silberberg, I., R. L. Baer, and S. A. Rosenthal.** 1976. The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. J Invest Dermatol. **66**(4):210-7.

189.**Silberberg-Sinakin, I., I. Gigli, R. L. Baer, and G. J. Thorbecke.** 1980. Langerhans cells: role in contact hypersensitivity and relationship to lymphoid dendritic cells and to macrophages. Immunol Rev. **53**:203-32.

190.**Simon, J. C., P. D. Cruz, Jr., R. E. Tigelaar, R. D. Sontheimer, and P. R. Bergstresser.** 1991. Adhesion molecules CD11a, CD18, and ICAM-1 on human epidermal Langerhans cells serve a functional role in the activation of alloreactive T cells. J Invest Dermatol. **96**(1):148-51.

191.**Sokolov, V. E.** 1982. Mammal Skin. University of California Press, Berkley and L.A.

192.**Sontheimer, R. D.** 1989. Perivascular dendritic macrophages as immunobiological constituents of the human dermal microvascular unit. J Invest Dermatol. **93**(2 Suppl):96S-101S.

193.**Sozzani, S., F. Sallusto, W. Luini, D. Zhou, L. Piemonti, P. Allavena, J. Van Damme, S. Valitutti, A. Lanzavecchia, and A. Mantovani.** 1995. Migration of dendritic cells in response to formyl peptides, C5a, and a distinct set of chemokines. J Immunol. **155**(7):3292-5.

194.**Sprent, J., Z. Cai, A. Brunmark, M. R. Jackson, and P. A. Peterson.** 1997. Constructing artificial antigen-presenting cells from Drosophila cells. Adv Exp Med Biol. **417**:249-54.

195.**Springer, T. A.** 1990. Adhesion receptors of the immune system. Nature. **346**(6283):425-34.

196.**Springer, T. A.** 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. **76**(2):301-14.

197.**Springer, T. A., D. Davignon, M. K. Ho, K. Kurzinger, E. Martz, and F. Sanchez-Madrid.** 1982. LFA-1 and Lyt-2,3, molecules associated with T lymphocyte-

mediated killing; and Mac-1, an LFA-1 homologue associated with complement receptor function. Immunol Rev. **68:**171-95.

198.**Staquet, M. J., C. Dezutter-Dambuyant, D. Schmitt, M. Amiot, L. Boumsell, and J. Thivolet.** 1989. A surface glycoprotein complex related to the adhesive receptors of the VLA family, shared by epidermal Langerhans cells and basal keratinocytes. J Invest Dermatol. **92**(5):739-45.

199.**Steinman, R. M.** 1991. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol. **9:**271-96.

200.**Stenn, K.** 1983. The Skin. Elsevier Science Publishing Co, New York.

201.**Stingl, G., E. Tschachler, V. Groh, K. Wolff, and C. Hauser.** 1989. The immune functions of epidermal cells. Immunol Ser. **46:**3-72.

202.**Streilein, J. W.** 1978. Lymphocyte traffic, T-cell malignancies and the skin. J Invest Dermatol. **71**(3):167-71.

203.**Streilein, J. W., and S. F. Grammer.** 1989. *In vitro* evidence that Langerhans cells can adopt two functionally distinct forms capable of antigen presentation to T lymphocytes. J Immunol. **143**(12):3925-33.

204.**Streilein, J. W., S. F. Grammer, T. Yoshikawa, A. Demidem, and M. Vermeer.** 1990. Functional dichotomy between Langerhans cells that present antigen to naive and to memory/effector T lymphocytes. Immunol Rev. **117:**159-83.

205.**Streilein, J. W., L. W. Lonsberry, and P. R. Bergstresser.** 1982. Depletion of epidermal langerhans cells and Ia immunogenicity from tape-stripped mouse skin. J Exp Med. **155**(3):863-71.

206.**Streilein, J. W., G. B. Toews, and P. R. Bergstresser.** 1979. Corneal allografts fail to express Ia antigens. Nature. **282**(5736):326-7.

207.**Streilein, J. W., G. T. Toews, J. N. Gilliam, and P. R. Bergstresser.** 1980. Tolerance or hypersensitivity to 2,4-dinitro-1-fluorobenzene: the role of Langerhans cell density within epidermis. J Invest Dermatol. **74**(5):319-22.

208.**Streilein, J. W., G. A. Wilbanks, and S. W. Cousins.** 1992. Immunoregulatory mechanisms of the eye. J Neuroimmunol. **39**(3):185-200.

209.**Strobl, H., C. Bello-Fernandez, E. Riedl, W. F. Pickl, O. Majdic, S. D. Lyman, and W. Knapp.** 1997. flt3 ligand in cooperation with transforming growth factor-beta1 potentiates *in vitro* development of Langerhans-type dendritic cells and allows single-cell dendritic cell cluster formation under serum-free conditions. Blood. **90**(4):1425-34.

210.**Symington, F. W., W. Brady, and P. S. Linsley.** 1993. Expression and function of B7 on human epidermal Langerhans cells. J Immunol. **150**(4):1286-95.

211.**Taglietti, M.** 1995. Vaccine adjuvancy: a new potential area of development for GM-CSF. Adv Exp Med Biol. **378**:565-9.

212.**Takashima, A., D. Edelbaum, T. Kitajima, R. K. Shadduck, G. L. Gilmore, S. Xu, R. S. Taylor, P. R. Bergstresser, and K. Ariizumi.** 1995. Colony-stimulating factor-1 secreted by fibroblasts promotes the growth of dendritic cell lines (XS series) derived from murine epidermis. J Immunol. **154**(10):5128-35.

213.**Takematsu, H., and H. Tagami.** 1990. Interleukin 2, soluble interleukin 2 receptor, and interferon-gamma in the suction blister fluids from psoriatic skin. Arch Dermatol Res. **282**(3):149-52.

214.**Tang, A., M. Amagai, L. G. Granger, J. R. Stanley, and M. C. Udey.** 1993. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. Nature. **361**(6407):82-5.

215.**Tao, M. H., and R. Levy.** 1993. Idiotype/granulocyte-macrophage colony-stimulating factor fusion protein as a vaccine for B-cell lymphoma. Nature. **362**(6422):755-8.

216.**Teunissen, M. B., H. A. Rongen, and J. D. Bos.** 1994. Function of adhesion molecules lymphocyte function-associated antigen-3 and intercellular adhesion molecule-1 on human epidermal Langerhans cells in antigen-specific T cell activation. J Immunol. **152**(7):3400-9.

217.**Teunissen, M. B., J. Wormmeester, S. R. Krieg, P. J. Peters, I. M. Vogels, M. L. Kapsenberg, and J. D. Bos.** 1990. Human epidermal Langerhans cells undergo profound morphologic and phenotypical changes during *in vitro* culture. J Invest Dermatol. **94**(2):166-73.

218.**Teunissen MBM, M. L. K., JD Bos.** 1997. Langerhans cells and related Skin dendritic cells, 2 ed. CRC Press, Boca Raton.

219.**Toews, G. B., P. R. Bergstresser, and J. W. Streilein.** 1980. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol. **124**(1):445-53.

220.**Trefzer, U., M. Brockhaus, H. Loetscher, F. Parlow, A. Kapp, E. Schopf, and J. Krutmann.** 1991. 55-kd tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. J Invest Dermatol. **97**(5):911-6.

221.**Van Kooyk, Y., A. de Vries-van der Zwan, L. P. de Waal, and C. G. Figdor.** 1994. Efficiency of antibodies directed against adhesion molecules to prolong skin graft survival in mice. Transplant Proc. **26**(2):401-3.

222.**Volc-Platzer, B., O. Majdic, W. Knapp, K. Wolff, W. Hinterberger, K. Lechner, and G. Stingl.** 1984. Evidence of HLA-DR antigen biosynthesis by human keratinocytes in disease. J Exp Med. **159**(6):1784-9.

223.**Walunas, T. L., D. J. Lenschow, C. Y. Bakker, P. S. Linsley, G. J. Freeman, J. M. Green, C. B. Thompson, and J. A. Bluestone.** 1994. CTLA-4 can function as a negative regulator of T cell activation. Immunity. **1**(5):405-13.

224.**Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Fodinger, J. P. Kinet, and G. Stingl.** 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc epsilon RI. J Exp Med. **175**(5):1353-65.

225.**Weiss, J. M., J. Sleeman, A. C. Renkl, H. Dittmar, C. C. Termeer, S. Taxis, N. Howells, M. Hofmann, G. Kohler, E. Schopf, H. Ponta, P. Herrlich, and J. C. Simon.** 1997. An essential role for CD44 variant isoforms in epidermal Langerhans cell and blood dendritic cell function. J Cell Biol. **137**(5):1137-47.

226.**Welsh, E. A., and M. L. Kripke.** 1990. Murine Thy-1+ dendritic epidermal cells induce immunologic tolerance *in vivo*. J Immunol. **144**(3):883-91.

227.**Williams, I. R., R. J. Ort, and T. S. Kupper.** 1994. Keratinocyte expression of B7-1 in transgenic mice amplifies the primary immune response to cutaneous antigens. Proc Natl Acad Sci U S A. **91**(26):12780-4.

228.**Wilmer, J. L., F. G. Burleson, F. Kayama, J. Kanno, and M. I. Luster.** 1994. Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. J Invest Dermatol. **102**(6):915-22.

229.**Winston, B. W., P. M. Krein, C. Mowat, and Y. Huang.** 1999. Cytokine-induced macrophage differentiation: a tale of 2 genes. Clin Invest Med. **22**(6):236-55.

230.**Witmer-Pack, M. D., W. Olivier, J. Valinsky, G. Schuler, and R. M. Steinman.** 1987. Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. J Exp Med. **166**(5):1484-98.

231.**Wolff, K., and G. Stingl.** 1983. The Langerhans cell. J Invest Dermatol. **80 Suppl**:17s-21s.

232.**Wolff, K., and R. K. Winkelmann.** 1967. Quantitative studies on the Langerhans cell population of guinea pig epidermis. J Invest Dermatol. **48**(6):504-13.

233.**Wolff, K., and R. K. Winkelmann.** 1967. Ultrastructural localization of nucleoside triphosphatase in Langerhans cells. J Invest Dermatol. **48**(1):50-4.

234.**Wong, G. H., and D. V. Goeddel.** 1986. Tumour necrosis factors alpha and beta inhibit virus replication and synergize with interferons. Nature. **323**(6091):819-22.

235.**Xu, L. L., M. K. Warren, W. L. Rose, W. Gong, and J. M. Wang.** 1996. Human recombinant monocyte chemotactic protein and other C-C chemokines bind and induce directional migration of dendritic cells *in vitro*. J Leukoc Biol. **60**(3):365-71.

236.**Yamazaki, S., H. Yokozeki, T. Satoh, I. Katayama, and K. Nishioka.** 1998. TNF-alpha, RANTES, and MCP-1 are major chemoattractants of murine Langerhans cells to the regional lymph nodes. Exp Dermatol. **7**(1):35-41.

237.**Ying, S., L. Taborda-Barata, Q. Meng, M. Humbert, and A. B. Kay.** 1995. The kinetics of allergen-induced transcription of messenger RNA for monocyte chemotactic protein-3 and RANTES in the skin of human atopic subjects: relationship to eosinophil, T cell, and macrophage recruitment. J Exp Med. **181**(6):2153-9.

238.**Zheng, P., and Y. Liu.** 1997. Costimulation by B7 modulates specificity of cytotoxic T lymphocytes: a missing link that explains some bystander T cell activation. J Exp Med. **186**(10):1787-91.

VITA

Pratima Deshpande

Candidate for the Degree of

Master of Science

Thesis: THE ROLE OF CYTOKINES IN LANGERHANS CELL MIGRATION FOLLOWING TREATMENT OF MURINE SKIN WITH ANTI-CD80 ANTIBODY

Major Field: Microbiology, Cell and Molecular Biology

Biographical:

Personal Data: Born in Hyderabad, India to Chandrakala Deshpande and Prabhaker Rao Deshpande.

Education: Graduated from Amrit Kapadia Navjivan, Hyderabad, India in 1992; received Bachelor of Science Degree in Genetics, Zoology and Chemistry from Osmania University, Hyderabad, India in May 1995 and a Bachelor of Science Degree (transfer) in Cell and Molecular Biology from Oklahoma State University, Stillwater, Oklahoma in December 1998. Completed the requirements for the Master of Science Degree at Oklahoma State University in December, 2001.

Experience: Tutor, Multicultural Development and Assessment Center, Oklahoma State University, January 1998 to April 1998; Lab Technician, employed by Dr. Kim Burnham, Department of Microbiology and Molecular Genetics, Oklahoma State University, May 1998 to December 1998; Teaching Assistant, Department of Microbiology and Molecular Genetics, Oklahoma State University, January 1999 to July 1998 and January 2001 to July 2001. Research Assistant, Department of Microbiology and Molecular Genetics, Oklahoma State University, August 1999 to December 1999. Lab Analyst, Food and Agricultural Products Center, Oklahoma State University, January 2000 to April 2000.

Affiliation: American Society for Microbiology.