The Use of Human T-Lymphocyte Clones to Study T-Cell Function in Allergic Contact Dermatitis to Urushiol

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Allergic contact dermatitis to poison ivy (Toxicodendron radicans) is believed to be mediated by T lymphocytes specific for the hapten urushiol. Activated T lymphocytes may produce pathology by a variety of mechanisms including direct cytotoxicity, production of lymphokines, recruitment of non-specific effector cells, non-specific cytotoxicity, and possibly autologous DR reactivity. The regulation and pathogenesis of this condition was studied by cloning and characterizing urushiol-specific T cells from the peripheral blood of patients with poison ivy dermatitis. Multiple CD8+ (T8+) urushiol-specific clones were derived. All clones that proliferated in response to a crude extract of T. radicans also proliferated in response to purified urushiol. Thus, urushiol appears to be the single immunogenic component of T. radicans resin. Pentadecylcatechol (PDC), which differs from urushiol only in the lack of unsaturated bonds in its lipophilic tail, stimulated only one of seven clones tested. This suggests that the double bonds in the C15 lipophilic tail of urushiol are required for antigenicity. Several of the CD8+ urushiol-specific clones suppressed pokeweed mitogen-induced IgG production in the presence of urushiol. Suppression was triggered specifically by urushiol and required MHC compatibility both for the antigen-presenting cells and the responding B cells. These suppressor clones were isolated from convalescent blood and may represent a mechanism for the termination of an allergic contact dermatitis. J Invest Dermatol 90:108S-111S, 1990

The pathogenesis of allergic contact dermatitis involves several steps. These stages include initial antigen presentation in skin and regional lymph nodes, homing of activated cells to the skin, recognition of antigen in the skin by specific T cells, and production of pathologic changes (Table I). T lymphocytes are involved at several of these stages in a variety of roles which may be dissected with the use of T-cell clones.

THE USE OF T-CELL CLONES IN THE STUDY OF DERMATOLOGY CONDITIONS

The basic principle behind cloning T cells is that activated T cells express the high-affinity receptor for IL2 [1]. Addition of IL2 to T cells will then induce proliferation. By specifically activating T cells with an antigen one can derive an antigen-specific T-cell line. Periodic re-stimulation with antigen plus autologous antigen-presenting cells is required to maintain both expression of high-affinity IL-2 receptors and antigen specificity. The T-cell line can then be cloned by limiting dilution or growth in soft agar.

Using the above principals, T lymphocytes have been cloned from tissues exhibiting a large variety of conditions, including the brains of multiple sclerosis patients [2], livers of chronic hepatitis patients [3], thyroids of patients with thyroiditis [4], rejecting renal allografts [5], and malignant neoplasms [6]. Robert Modlin and Ottenhoff et al [7,8] have cloned T cells from skin lesions of both lepromatous and tuberculoid leprosy. Leproni specific suppressor T cells were isolated from lesions of lepromatous leprosy and helper T cells were isolated from lesions of tuberculoid leprosy [9]. Clones isolated from lesions of tuberculoid leprosy were used to characterize the relevant antigens of M. leprae. Nickel- [10] and cobalt- [11] specific T cells have also been cloned from both skin and blood of patients with allergic contact dermatitis to these substances.

INITIAL PRESENTATION OF HAPten (URUSHIOL)

Urushiol is the antigenic hapten responsible for allergic contact dermatitis to poison ivy (Toxicodendron radicans) [12]. It consists of a catechol nucleus with a C15 lipophilic tail containing 2–3 unsaturated bonds [13–15]. The conjugation of urushiol to cells is believed to involve both an initial lipophilic interaction and a covalent bond with proteins through a quinone intermediate [16]. The purified urushiol used for my studies was a generous gift of Dr. H. Baer (Burke Lab of Biologics, Food and Drug Administration, Bethesda, MD). Analysis of the purified urushiol by gas chromatography showed no saturated side chains and a minimum of 95% catechol (Dr. Baer, personal communication). The material was 85.1% C15 triene, 10.2% combination mono and diene, and 4.7% C17 triene. The hapten Urushiol binds to epidermal cells, both keratinocytes and Langerhans cells. Langerhans cells conjugated with urushiol may then migrate through lymphatics to lymph nodes, where they present antigen to T cells [17]. Keratinocytes may also have a role in initial recognition of antigen. Keratinocyte “activation” may result from a number of stimuli, including injury and UVB irradiation, and may represent an early
Table I. Stages in the Pathogenesis of Allergic Contact Dermatitis

1. Binding of hapten to Langerhans cells
2. Migration of Langerhans cells to lymph nodes
3. Initial antigen presentation by Langerhans cells
4. Homing of activated T lymphocytes to skin
5. Recognition of antigen in skin
6. Pathology induced by a variety of effector mechanisms

event in the recruitment of leukocytes. Activated keratinocytes produce many cytokines including IL-1, IL-6, and GM-CSF [18–20]. These cytokines may have relevance to enhanced antigen presentation, T-cell activation, or homing. Production of these cytokines can be stimulated by IL-1 through an autocrine pathway regulated in part by expression of high-affinity receptors for IL-1 [18].

Recognition of antigen is in the context of major histocompatibility antigens (MHC). CD4 (T4) cells recognize antigen in association with class II MHC (I-DR and I-DB T8) cells recognize antigen in association with class I MHC (HLA-A,B,C) [21]. The MHC class I antigen has been studied by x-ray diffraction [22]. It was found that the molecule has an antigen-presentation groove capable of binding peptides. The T-cell receptor is believed to interact simultaneously with both the MHC molecule and the peptide held within the groove [23].

HOMING OF ANTIGEN-SPECIFIC CELLS TO SKIN

Activated T cells must then enter the site of inflammation. T cells have specific homing receptors for high endothelial venules (HEV) of peripheral lymph nodes or mucosal lymphatic tissue. These endothelial homing receptors are characterized by the lymphocyte receptors MEL-14 for mouse peripheral lymph nodes [24] and Hermes-3 (CD44) which recognizes human mucosal HEV [25]. Homing receptors interact with an endothelial ligand or “addressin.” Murine addresses include MECCA 367 [26], which is present on mucosal HEV, and MECCA 79, which is present on peripheral lymph-node HEV [27].

Skin post-capillary venules probably develop features of HEV in sites of chronic inflammation and the skin may represent a distinct homing specificity. It is possible to demonstrate this homing in vitro by adherence of lymphocytes to frozen tissue sections of lymph nodes [28]. Similar binding of lymphocytes to frozen skin sections has been demonstrated by Yee Chin’s laboratory [29]. Lymphocytes will only bind to diseased but not to normal skin. However, gamma-interferon treatment of skin explants will induce lymphocyte adherence [30]. This has been shown to result from the induction of ICAM-1 on endothelial cells. ICAM-1 is the ligand for LFA-1, a lymphocyte adhesion molecule critical in most lymphocyte cell interactions [31,32]. Gamma-interferon has been shown to induce ICAM-1 expression on keratinocytes and endothelial cells [33].

RECOGNITION OF ANTIGEN IN THE SKIN

Langerhans cells are the principal antigen-presenting cells of the skin [34]. CD4+ lymphocyte recognition of antigen requires class II major histocompatibility complex antigen (MHC), which is present on Langerhans cells but may be induced on keratinocytes by gamma-interferon [35]. CD8+ T-cell recognition of antigen does not require cells to be class II MHC positive but is dependent upon recognition of antigen with class I MHC. In vivo data suggests that recognition of antigen in association with class II MHC positive keratinocytes results in down-regulation of the immune response [36].

EFFECTION MECHANISMS

Upon reaching the skin and recognizing the antigen, T cells may induce pathology by a variety of possible mechanisms. These include lymphokine production, recruitment of non-specific effector cells, antigen-specific cytotoxicity, and non-specific cytotoxicity. Lymphokine production by activated lymphocytes may be significant to the pathogenesis of allergic contact dermatitis. These lymphokines include IL-2, IL-4, IL-6, lymphotixin, gamma-interferon, GM-CSF, and MIF [37]. Gamma-interferon has multiple effects. In addition to an anti-viral action, gamma-interferon activates macrophages and induces an increased expression of a variety of cell-surface proteins, such as MHC class I, MHC class II, and ICAM-1 [33,35]. Increased MHC class I and II expression is of significance to antigen presentation and recognition. ICAM-1 expression is important to lymphocyte homing and lymphocyte keratinocyte interactions [29,30]. In addition, through the action of lymphokines, it is possible for T cells to recruit macrophages and neutrophils.

T cells are also capable of direct cell-mediated cytotoxicity. Recognition of target cells is dependent upon co-recognition of MHC structures. Because most nucleated cells have MHC class I molecules but not class II molecules, CD8+ cells are the primary cytotoxic cell. Cell-mediated cytotoxicity is probably important in the control of viral infection and in conditions with lichenoid infiltrates and basal cell damage (e.g., lichen planus) as well as conditions with satellite cell necrosis (e.g., graft-vs-host disease). However, the role of cell-mediated cytotoxicity in allergic contact dermatis is uncertain.

Activated lymphocytes are also capable of non-specific cytotoxicity. This is defined as the ability to kill a variety of target cells without prior sensitization. Natural killer (NK) cells are capable of killing a variety of leukemic cell lines without in vitro activation [38]. Lymphokine-activated killer (LAK) cells kill a wide variety of tumor and normal cells that are resistant to NK cytotoxicity [39]. LAK cells are generated by incubation of lymphocytes with high concentrations of interleukin-2 (IL-2). Both NK and LAK cells are heterogeneous and should be regarded as activities rather than distinct cell types.

Human keratinocytes can be killed in vitro by LAK cells using a standard 4-h 51chromium release assay [40]. Prior treatment of the keratinocytes with gamma-interferon will both induce ICAM-1 expression and increase lysis by LAK cells. This increased lysis of keratinocytes can be blocked by antibodies to either ICAM-1 or LFA-1 but not by antibodies to CD4, CD8, MHC class I, or MHC class II. It is hypothesized that production of gamma-interferon by activated lymphocytes may sensitize keratinocytes to lysis as “innocent bystanders” during an immune response to a non-epidermal antigen.

Finally, there is evidence that normally occurring autoreactive T cells may amplify pathology by recognizing autologous MHC class II (DR) antigen. Gamma-interferon induces DR on keratinocytes and DR+ keratinocytes are present in many disorders, particularly those with a lichenoid infiltrate. Saito et al. [41] have shown that intradermal injection of mice with auto-class II MHC (Ia) reactive T-cell clones induces skin lesions resembling lichen planus.

CLONING OF URUSHIOL-SPECIFIC T CELLS

In an effort to determine the relevant effector mechanisms in allergic contact dermatitis to poison ivy, urushiol-specific T cells were cloned from peripheral blood [42,43]. Peripheral blood lymphocytes from urushiol-sensitive donors were sensitized in vitro with a DMSO extract of T. radicans leaves. After 7 d, IL-2 containing conditioned medium was added to the cultures. The resulting T-cell line was tested for proliferation to T. radicans extract and cloned by limiting dilution with antigen and autologous antigen-presenting cells. Multiple urushiol-specific clones were generated from peripheral blood. Six such clones have been extensively characterized [42,43]. Most of the characterized urushiol-specific clones have been CD8+ or CD4+CD8+. The double-positive CD4+CD8+ clone was found to be class I MHC restricted [42]. Thus, it functioned as a CD8+ clone. This finding implies that the presence of the CD4 molecule alone is insufficient to determine MHC class restriction. Rather it is likely that the T-cell receptor, which recognizes antigen in association with MHC, determines the MHC class restriction. The predominance of CD8+ clones was not an artifact of the technique, as numerous CD4+ autoreactive and tetanus toxoid-specific clones were derived from the same donors using the same procedure. Recently, CD4+ CD8-urushiol-specific clones
Figure 1. Proliferation of T-lymphocyte clones in response to a variety of stimuli. Cloned T cells were added to microtiter wells (1 × 10^6 cells/well) along with equal numbers of PBMC used as antigen-presenting cells (APC). Designated wells received in addition either pure urushiol, DMSO extract of *T. radicans* leaves (RHUS), or pentadecylcatechol (PDC). MEDIA wells did not receive APC. 3H-thymidine was added at 24 h and plates were harvested 15 h later. Quadruplicate wells were used for each condition. SEM was under 10% of total dpm.

have also been isolated from the convoluted peripheral blood of this same donor.

The isolation of CD8+ urushiol-specific T-cell clones was unexpected. Prior studies have found that the response to nickel [10] and cobalt in sensitive patients is mediated by CD4+ cells [11]. CD4+ cells were cloned from both peripheral blood and skin lesions of patients with allergic contact dermatitis to these substances. These clones were heterogeneous with regard to antigen-presenting cell requirements. Many clones responded to antigen presented by either peripheral blood mononuclear cells or epidermal dendritic cells (Langerhans cells), whereas other clones only proliferated in response to antigen presented by Langerhans cells [44]. The experimental literature on the response to hapten in mice also indicates a requirement for CD4+ inducer cells.

Recognition of an antigen by CD4+ vs CD8+ cells is now thought to reflect the cellular processing of the antigen. Soluble, exogenous protein antigens appear to preferentially associate with MHC class II molecules and interact with CD4+ cells. In contrast, proteins synthesized within the cell (e.g., viral, tumor associated, and transplantation antigens) tend to associate with MHC class I molecules and interact with CD8+ cells [45]. Recognition of urushiol by CD8+ cells suggests that it fits into the antigen-presentation pathway for internally synthesized antigens. This may reflect the processing of the cell components to which urushiol binds.

Many clones were cytotoxic in the presence of Con A (lectin-dependent cytotoxicity) but cytotoxicity for urushiol-conjugated targets was not detected. In the absence of a positive control, it is uncertain whether this lack of cytotoxicity reflects the degree of urushiol conjugation. Several clones produced significant levels of both gamma-interferon and IL-2. The maximum levels of IL-2 (5 U/ml for 1 × 10^6 cells/ml) and gamma-interferon (120 U/ml) were less than that reported for CD4+ clones under similar conditions [10,11].

**USE OF T-CELL CLONES TO CONFIRM THAT URUSHIOL IS THE SOLE ANTIGENIC COMPONENT OF POISON IVY (T. RADICANS)**

The above T-cell clones were all derived by in vitro secondary stimulation with a crude DMSO extract of *T. radicans* leaves. The clones were subsequently screened for proliferation to crude leaf extract, pure urushiol, pentadecylcatechol (PDC), and an irrelevant antigen (tetanus toxoid) to which the donor was also sensitive (Fig 1). All clones which responded to crude leaf extract also responded to pure urushiol. Only one of seven urushiol responsive clones tested also responded to PDC. This confirms at a clonal level that PDC is only weakly cross-reactive with urushiol in humans. PDC differs from urushiol only in that the lipophilic C15 tail is fully saturated, whereas the C15 tail of urushiol contains two or three unsaturated bonds. The lack of antigenicity of PDC suggests that the C15 tail interacts with a hydrophobic domain with specific conformational restraints.

**URUSHIOL-SPECIFIC SUPPRESSOR CLONES**

Urushiol-specific T-cell clones were also tested for their ability to help or suppress pokeweed mitogen (PWM)-induced IgG production (Fig 2). No helper activity was detected. However, several clones were able to suppress IgG production.

One such clone, RL9-7, was extensively characterized (Fig 2) [42]. This clone was CD3+CD4+CD8+WT31+, but class I MHC restricted. WT31 is a monoclonal antibody which reacts with the α/β T-cell receptor. RL9-7 proliferated specifically to both urushiol and crude poison ivy extracts, but not to tetanus toxoid or pentadecylcatechol. This clone suppressed PWM-induced IgG production, but only in the presence of urushiol. Thus, RL9-7 was triggered in an antigen-specific manner to suppress a non-antigen-specific response. However, even in the presence of autologous antigen-presenting cells, the clone was unable to suppress allogeneic cells. This indicates a genetic restriction on the interaction between RL9-7 suppressor cells and the target B cell.

The above suppressor clones were all derived from peripheral blood several months following an acute eruption to urushiol. It is possible that these suppressor clones represent a mechanism for the termination of the immune response to a contact allergen.

**CONCLUSION**

Cloning of T cells from lymphocyte-mediated dermatologic conditions represents a powerful tool for the study of these diseases. Characterization of such clones should help delineate the effector and regulatory mechanisms of lymphocyte-mediated pathology, as well as helping to define the relevant antigens.

**REFERENCES**


