

Mononuclear cell subpopulations and infiltrating lymphocytes in erythema dyschromicum perstans and vitiligo

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Summary. Erythema dyschromicum perstans (EDP) and vitiligo are two cutaneous pigmentary dermatoses of unknown etiology. In the present study, the leukocyte infiltrates in the affected skin of EDP and vitiligo patients were studied using the avidin-biotin (ABC) immunoperoxidase technique and monoclonal antibodies which recognise the following mononuclear cell subgroups: T-suppressor/cytotoxic (CD8-Leu-2), T-helper (CD4 = OKT4), T-suppressor + macrophages (Leu-15), Pan T (CD3 = Leu-4), macrophages (Leu-M3) and Langerhans cells (CD1 = Leu-6), and other cellular markers such as Ia antigens and the Interleukin-2 receptor (CD25 = TAC). The immunocytochemical analysis showed a selective accumulation of CD3+, CD8+, Leu-15-, T-cytotoxic cells in the epidermis of both EDP and early lesions of vitiligo. In addition, an increase in the number of epidermal Langerhans cells (CD1+) was observed in some cases of EDP and vitiligo. The CD4/CD8 ratios in affected and uninvolved skin for both disorders were not significantly different, although values lower than unity were only observed in the infiltrates of affected skin. Ia antigen positivity was observed in the dendritic cells of the dermis and epidermis, as well as in most of the lymphoid cells within the infiltrates for both diseases. Macrophages (Leu-M3) in EDP dermal infiltrates were generally found adjacent to extracellular melanin pigment. Lymphocytes expressing TAC (CD25) surface antigens were also present in the dermal infiltrates. These morphological observations suggest a possible immune cell participation in the dyschromia of such cutaneous disorders.

Key words: Immune cells - Erythema dyschromicum perstans - Vitiligo - Avidin biotin immunoperoxidase

Introduction

Erythema Dyschromicum Perstans (EDP) is an

uncommon disorder characterised by persistent, ash-grey, macular discolouration of the skin often associated with an active polycyclic outline (Convit, Kerdel-Vegas & Rodríguez, 1961). Most patients with this disease are Latin Americans (Ramírez, 1957; Convit et al., 1961; Ramirez, 1966), although cases have been described in other parts of the world (Stevenson and Miura, 1966; Knox, Dodge and Freeman, 1968). Histological features include a hydropic degeneration or liquefaction of the epidermal basal layer, which explains the «incontinentia pigmenti» in the dermis. The melanin is phagocytised by macrophages, causing the characteristic colouration. Other features include edema of the dermal papillae and perivascular infiltrates of variable intensity in the papillar and subpapillar dermis, constituted mainly by histiocytes and lymphocytes. Occasionally, colloid bodies or Civatte bodies are seen (Convit et al., 1961; Knox et al., 1968). Characteristic nonspecific ultrastructure includes desmosome retraction, vacuolisation and dermal melanophagocytosis (Soter, Wand and Freeman, 1969).

Vitiligo is another skin disorder characterised by depigmented patches, which are irregularly defined and are often surrounded by hypopigmented skin (Nordlund and Lerner, 1979). The essential process in vitiligo is the progressive destruction of melanocytes (Brown, et al., 1967; Morohashi et al., 1977). The presence of mild dermal lymphocytic infiltrates at the border of the depigmented areas has been described (Bazek et al., 1977; Nordlund et al., 1980). In addition, variations in the number of Langerhans cells in the vitiliginous epidermis have been reported (Birbeck et al., 1961; Riley, 1967; Nordlund and Lerner, 1979). Ultrastructural studies have confirmed the absence of melanocytes in areas of long standing vitiligo (Birbeck et al., 1961) and melanocyte degeneration in surrounding hypopigmented areas (Morohashi et al., 1977). The pathogenic mechanisms of both EDP and vitiligo are unclear.

Since EDP and vitiligo have been associated with immune dysfunction (Knox et al., 1968; Nordlund and Lerner, 1979), we studied the possible involvement of

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immune cells by analysing the mononuclear cell subgroups in the skin of patients with either disease, using monoclonal antibodies and an immunoperoxidase technique.

Materials and methods

Patients:

Both EDP ($n = 10$) and vitiligo ($n = 5$) patients were fully characterised according to established clinical and histological criteria (Lerner, 1959; Pinkus, 1959; Convit et al., 1961). The duration of the disease ranged from 10 months to 6 years in EDP and from 2 weeks to 4 years in vitiligo. The mean \pm SD age was 33 ± 24 years in EDP and 31 ± 22 years in vitiligo.

Surgical (5×10 mm) and punch biopsies (5 mm in diameter) were taken from affected and uninvolved skin under local anesthesia (2% xylocaine). Biopsies from uninvolved skin were usually taken from a distant area, not-exposed to sunlight. Skin biopsies ($n = 4$) from normal volunteers with no evident skin disorder were also taken. For the immunoperoxidase studies, the skin biopsies were embedded in OCT compound (Lab Tek, Miles Labs. Inc., U.S.A.), snap-frozen in liquid nitrogen and stored at -20°C until examination.

Monoclonal antibodies:

All the well-characterised monoclonal antibodies used were diluted in a modified phosphate buffered saline, pH 7.2 (PBS) (Hofman et al., 1982). These antibodies recognised mononuclear cell markers for the following subpopulations (Bernard et al., 1984): T-suppressor/cytotoxic (CD8 = Leu-2, dil. 1:100), Pan T (CD3 = Leu-4, dil. 1:100), Langerhans cells (CD1 = Leu-6, dil. 1:100), T-suppressor + macrophages (Leu-15, dil. 1:25), macrophages (Leu-M3, dil. 1:50), purchased from Becton Dickenson, Inc., U.S.A.; T-helper (CD4 = OKT4, dil. 1:50), purchased from Ortho Diagnostics, Inc., U.S.A.; HLA-DR (Ia) (I2, dil. 1:400), purchased from Coulter Clone Inc., U.S.A.; Interleukin-2 receptor (CD24 = TAC, dil. 1:5000) donated by Dr. S. Gillis (Immunex Corp, Seattle, U.S.A.).

Immunoperoxidase staining procedure:

Frozen sections (5 μm) were cut with a cryostat and air-dried overnight before the immunostaining procedure. Some sections were also stained for haematoxylin-eosin (H&E).

Immunostaining was performed using the avidin-biotin (ABC) immunoperoxidase technique (Hsu et al., 1981), the sections being treated as follows: 1) Fixation in fresh acetone, 10 min.; 2) PBS, 5 min; 3) primary monoclonal antibody (optimal dilution), 15 min; 4) PBS, 5 min; 5) Biotinylated horse-anti mouse IgG (Vector Labs. Inc., U.S.A.) 1:30 in PBS (50 $\mu\text{g}/\text{ml}$), 15 min; 6) PBS, 5 min; 7) ABC (Vectastain kit, Vector Labs, Inc., U.S.A.) 1:100, 15 min; 8) PBS, 5 min; 9) Developing for

10 min with 90 μM H_2O_2 and 3-amino-9-ethyl-carbazole (final concentration 0.88 mM) which was dissolved in 50 mM N, N-dimethylformamide in 0.1M acetate buffer, pH 5.2; 10) Rinse in water; 11) Counterstaining with Mayer's haematoxylin and mounting in glycerin-gelatin.

Mononuclear cell quantification:

Cell counting was carried out using a light microscope with millimetered scale (Carl Zeiss, Germany), calibrated to determine the number of cells/ mm^2 in dermal infiltrates or epidermis. Only cells showing red-brownish immunostaining and a visible nucleus were counted as positive. Percentages of each phenotype in the dermal infiltrates were calculated according to Modlin et al. (1984) by assuming that there are approximately 3600 cells/ mm^2 of infiltrate.

Statistical analysis:

The cell counts were expressed as mean \pm SD per mm^2 of dermal infiltrates or epidermis.

Statistical analysis was performed using Wilcoxon's rank sum test.

Results

The histological analysis of skin biopsies of the two patient groups confirmed the clinical diagnosis of EDP or vitiligo.

The immunocytochemical study showed a high density of mononuclear cells bearing the phenotypes CD3+, CD8+, CD4+, and Leu-M3+ in the dermal infiltrates of patients with EDP, and this was statistically significantly elevated when compared with normal skin (Table 1). Vitiligo lesions similarly showed high densities of mononuclear cells bearing the phenotype CD4+, CD8+, CD3+ except for Leu-15 (Table 2). T-lymphocytes bearing the CD8+ phenotype tended to be localised in the periphery of the infiltrates, whereas CD4+ lymphocytes were usually distributed throughout (Figure 1a,b). The most significant observation in lesions of EDP, and recently depigmented skin of vitiligo, was a selective accumulation of T-lymphocytes CD3+, CD8+, Leu-15- in the epidermis, mainly in the basal layer towards the Malpighian layer (Figs. 2, 3). This selective accumulation of T-cells was not observed in normal skin (Tables 1 and 2). Leu-15+ cells (lymphocytes and macrophages) were only observed in the dermal infiltrates of both disorders (Fig. 4).

In addition, a significant increase in the number of epidermal Langerhans cells CD1+ was observed in EDP lesions (Table 1). In vitiligo, despite finding no significant differences in the mean numbers of Langerhans cells between lesions and normal skin (Table 2), some individual cases presented an apparent increase in these in the affected skin (Fig. 5, Table 3). In EDP there were also dendritic CD1+ cells within the dermal infiltrates, generally adjacent to the melanin pigment (Fig. 6).

The CD4/CD8 ratios for the infiltrates of both

Table 1. Mononuclear cell densities (mean \pm standard deviation) in lesions of Erythema Dyschromicum Perstans and normal control skin. (NS).

	Phenotype	EDP (cells/mm ²)	NS (cells/mm ²)	p
Dermal infiltrates:	CD4+ = OKT4	1286 \pm 225	845 \pm 256	< 0,05
	CD8 + = Leu-2+	1186 \pm 355	594 \pm 302	< 0,01
	Leu-15+	879 \pm 99	267 \pm 26	< 0,05
	CD3+ = Leu-4	1589 \pm 375	819 \pm 441	< 0,05
	Leu-M3	405 \pm 153	344 \pm 85	nss
	CD4/CD8	1,23 \pm 0,37	1,52 \pm 0,31	nss
Epidermis:	CD1+ = Leu-6+	1084 \pm 905	658 \pm 265	< 0,05
	CD8+, CD3+, Leu-15-	113 \pm 85	4 \pm 6	< 0,05

nss = non statistical significant

Table 2. Mononuclear cell densities (mean \pm standard deviation) in lesions of Vitiligo and normal control skin (NS).

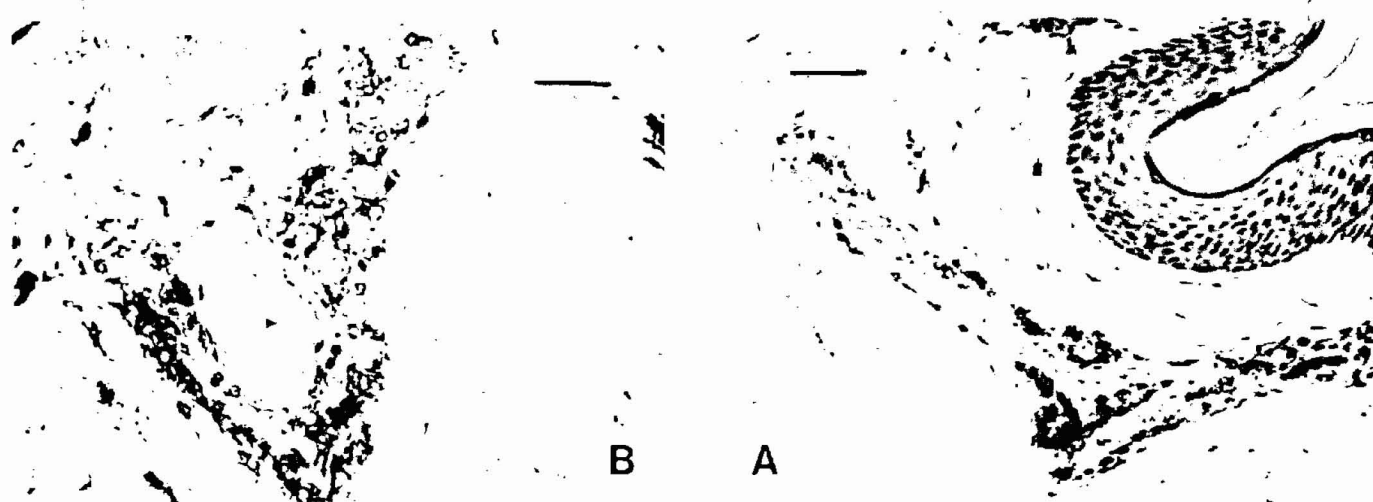
	Phenotype	Vitiligo (cells/mm ²)	NS (cells/mm ²)	p
Dermal infiltrates:	CD4+ = OKT4	711 \pm 318	845 \pm 256	< 0,05
	CD8 + = Leu-2+	727 \pm 491	594 \pm 302	nss
	Leu-15+	571 \pm 135	267 \pm 26	< 0,05
	CD3+ = Leu-4+	860 \pm 391	819 \pm 441	nss
	Leu-M3	381 \pm 185	344 \pm 85	nss
	CD4/CD8	1,29 \pm 0,69	1,52 \pm 0,31	nss
Epidermis:	CD1+ = Leu-6+	857 \pm 333	658 \pm 265	nss
	CD8+, CD5+, Leu-15-	40 \pm 22	4 \pm 6	< 0,05

nss = non statistical significant

Table 3. Langerhans cell numbers in the epidermis of vitiligo lesions

Patient	cells/mm ² of epidermis
1	555
2	468
3	1067
4	982
5	1224

Fig. 1. A CD8 staining in erythema dyschromicum perstans infiltrate. The cells staining positively for the suppressor/cytotoxic phenotype appear as small dark rings, and tend to be localized towards the periphery of the infiltrate. $\times 500$, scale bar = 20 μ m. B. CD4 staining in erythema dyschromicum perstans infiltrate. The cells bearing the T-helper/inducer phenotype (small dark rings) are distributed throughout the infiltrate. $\times 500$, scale bar = 20 μ m.



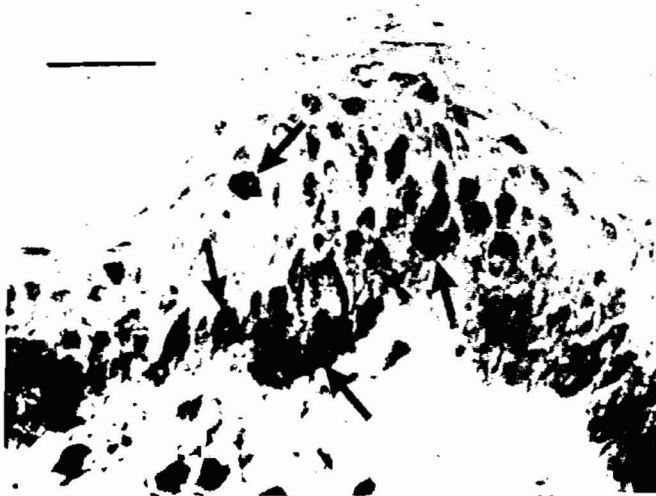


Fig. 2. CD8 staining in the epidermis of an erythema dyschromicum perstans lesion. Positive cells (arrows) are observed in the basal and Malpighian layers. $\times 1400$, scale bar = $10 \mu\text{m}$.



Fig. 3. CD8 staining in the epidermis of an early lesion (4 months) of vitiligo. A selective accumulation of T-suppressor/cytotoxic cells (CD8+) is observed in the epidermal basal layer and in the dermis. $\times 1400$, scale bar = $10 \mu\text{m}$.



Fig. 4. Leu-15 staining in a vitiligo lesion. The Leu-15+ cells (arrows) are only present within the dermal infiltrates. Some reactivity is observed in epidermal dendritic cells. $\times 530$, scale bar = $20 \mu\text{m}$.



Fig. 5. CD1 staining in the epidermis of vitiliginous skin. Langerhans cell bodies and dendrites are clearly immunostained. $\times 1400$, scale bar = $10 \mu\text{m}$.



Fig. 6. CD1 staining in the dermis of erythema dyschromicum perstans. The cells bearing the CD1 phenotype (arrows) are generally adjacent to the melanin pigment (arrow heads). $\times 1400$, scale bar = $10 \mu\text{m}$.

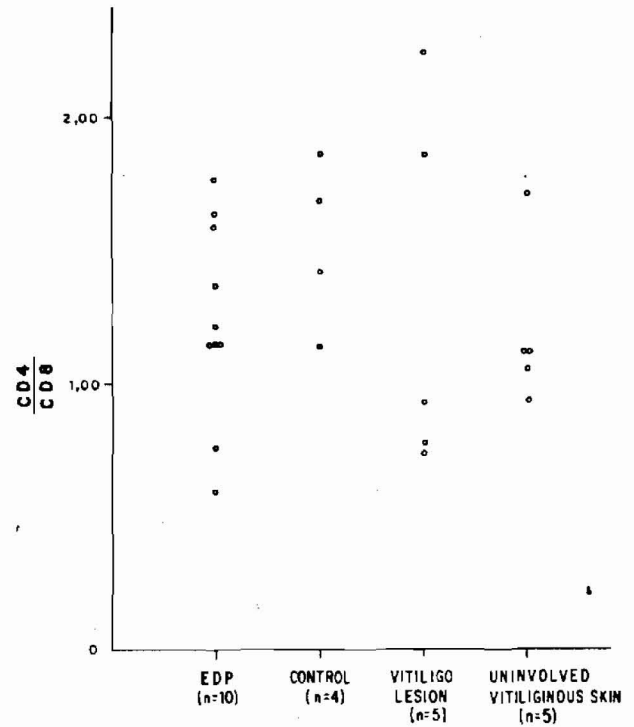


Fig. 7. T-helper/T-suppressor cell ratios in patients with erythema dyschromicum perstans, vitiligo, uninvolved vitiliginous skin and normal control skin.

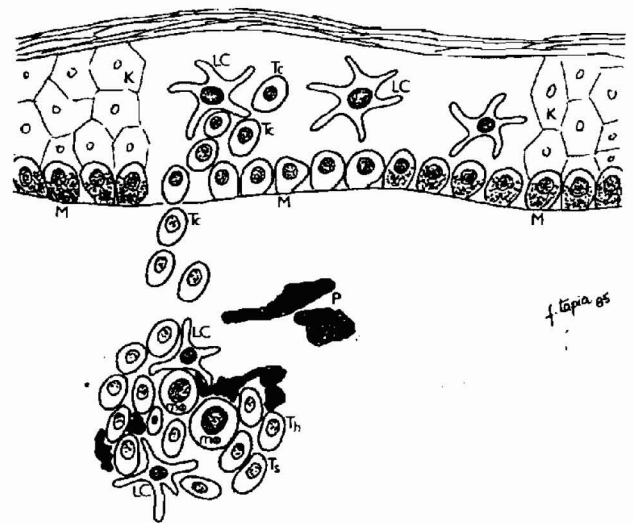


Fig. 8. A hypothesis for the immunological nature of various epidermal disorders:

1. T-lymphocytes are present in normal skin, and have an immunosurveillance function.
 2. An epidermal disorder could cause a subgroup of Langerhans cells and/or macrophages to interact with a subgroup of T cells.
 3. After the development of an immune response in the form of a dermal infiltrate, T cells of the cytotoxic subset migrate to the epidermis, where they attack and/or destroy the melanocytes or other cell types.
- LC = Langerhans cells, Tc = T-cytotoxic cells, M = melanocyte, m = macrophages, Ts = T-suppressor cells, Th = T-helper cells, K = keratinocytes.

disorders, and normal skin, were not significantly different (Fig. 7, Tables 1 and 2). However, values lower than unity were observed only in 20% of the patients with vitiligo and in 75% of those with EDP. Ia+ (HLA-DR+) immunoreactivity was observed in dendritic cells of the epidermis and dermis in the lesions of both diseases, as well as in most of the lymphoid cells present within the infiltrates.

Macrophages (Leu-M3+) occupied approximately 10% of the infiltrate in both disorders (Tables 1, 2).

Lymphocytes expressing the IL-2 receptor (TAC) were present in about 7% of the dermal infiltrates of both diseases and only occasionally in normal skin.

A comparison of the mononuclear cell densities between EDP and vitiligo showed statistically significant differences in the number of cells positive for the phenotypes CD3+, Leu-15+ and CD4+; these being higher in EDP than in vitiligo (Tables 1, 2). Although a comparison between the number of CD8+, Leu-15-cells present in the epidermis of both conditions did not show a statistically significant difference, these cells tended to be found in higher numbers in the epidermis of EDP lesions than in those of vitiligo (Tables 1,2).

Discussion

The presence of T-lymphocytes bearing the T-suppressor/cytotoxic phenotype at the dermo-epidermal junction, and within the epidermis has been described in early lesions of psoriasis (Bjerke, 1982; Hammar et al., 1984), lupus erythematosus (Bjerke, 1982), lichen planus (Bjerke, 1982; De Panfilis et al., 1983; Buechner, 1984), atopic dermatitis (Rocha et al., 1984) and in the duodenal epithelium in dermatitis herpetiformis (Ljunghall et al., 1982). Similarly, such T cells have also been observed in the epidermis of normal skin (Bjerke, 1982) and gut epithelium (Jannosy et al., 1980), suggesting that they may be epitheliotropic. This possibility indicates a physiological role for such cells as a surveillance mechanism, which, however, upon activation by a yet unknown stimulus might participate in the pathogenic process which alters the melanocytes or other epidermal cells. This study showed a selective accumulation of CD8+ T-lymphocytes in the basal epidermal layer of the two non-related skin disorders examined. Furthermore, the use of a newly described monoclonal antibody, Leu-15, which permits the differentiation of T-suppressor and T-cytotoxic subpopulations (Landay et al., 1983; Clement et al., 1984; Clement et al., 1984), allowed the identification of the epidermal CD8+ cells as T-cytotoxic lymphocytes, since Leu-15+ cells were not observed in the epidermis.

The finding that there are more CD3+, CD4+ T-cells in EDP than in vitiligo might suggest that a stronger immune process occurs in EDP. This is also indicated by the histopathological features of the disease.

The presence of Ia+ (HLA-DR+) cells and T cells expressing the IL-2 receptor (TAC+) provides a strong indication of ongoing immune activity within the infiltrates of EDP and vitiligo. If we accept the skin as a

major immunological organ (Streilein, 1983), the presence of T-cytotoxic cells in the normal epidermis and their increase in different skin disorders suggest the possible importance of this cell subgroup in the early stages of the immune response. We propose a possible immunological model (Fig. 8) to explain the possible sequence of events occurring in the diseases studied, where epidermal cells are involved in the pathology.

Our observations suggest that a possible cause of the dyschromia in lichenoid diseases is a factor, or a group of factors, as yet to be characterised, which acts locally, reversing the normal unresponsiveness to melanocyte antigens. This possibility is supported by the finding of antibodies against melanocytes in the sera of patients with vitiligo (Naughton et al., 1983). We suggest an important participation of Langerhans cells as antigen-presenting cells, and T-cytotoxic cells as effector cells, that could selectively damage the melanocytes.

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References

- Bazek A., Balas D. and Bazek J. (1977). Maladie de Vogt-Koyanagi-Harada. *Ann. Dermatol. Venereol.* 104, 849-853.
- Bernard A., Berstein I., Boumsell L., Dausset J., Evans R., Hansen J., Haynes B., Kersey J., Knapp W., McMichael A., Milstein C., Reinherz E., Ritts R.E. and Schlossman S.F. (1984). Committee on human leukocyte differentiation antigens: IUIS-WHO Nomenclature Subcommittee. *Bull. W.H.O.* 5:809-811.
- Birbeck M.S., Breathnach A.S. and Everall J.D. (1961). An electron microscope study of basal melanocytes and high level clear cells (Langerhans cells) in vitiligo. *J. Invest. Dermatol.* 37, 51-64.
- Bjerke J.R. (1982). Subpopulations of mononuclear cells in lesions in psoriasis, lichen planus and discoid lupus erythematosus studied using monoclonal antibodies. *Acta Dermatol. Venereol. (Stockn)*, 62, 477-483.
- Brown J., Winkelman R.K. and Wolff K. (1967). Langerhans cells in vitiligo. *J. Invest. Dermatol.* 49, 386-390.
- Buechner S.A. (1984). T cell subsets and macrophages in lichen planus. In situ identification using monoclonal antibodies and histochemical techniques. *Dermatologica* 169, 325-329.
- Clement L.T., Grossi C.E. and Gartland G.L. (1984). Morphologic and phenotypic features of the subpopulation of Leu-2+ cells that suppresses B cell differentiation. *J. Immunol.* 133, 2461-2468.
- Clement L.T., Dagg M.K. and Landay A. (1984). Characterization of human lymphocyte subpopulations: alloreactive cytotoxic T-lymphocyte precursor and effector cells are phenotypically distinct from Leu-2+ suppressor cells. *J. Clin Immunol.* 4, 395-402.
- Convit J., Kerdel-Vegas F. and Rodriguez G. (1961). Erythema

- dyschromicum perstans. A hitherto undescribed skin disease. *J. Invest. Dermatol.* 36, 457-462.
- De Panfilis G., Manara G.C., Ferrari C., Manfredi G. and Allegra F. (1983). Imbalance in phenotypic expression of T cell subpopulations during different evolutionary stages in lichen planus lesions. *Acta Dermatol. Venereol. (Stockn)* 63, 369-375.
- Hammar H., Gu S.Q., Johannesson A., Sundkvist K.G. and Biberfeld P. (1984). Subpopulations of mononuclear cells in microscopic lesions of psoriatic patients. Selective accumulation of suppressor/cytotoxic T cells in epidermis during the evolution of the skin. *J. Invest. Dermatol.* 83, 416-420.
- Hofman F.M., Billing R.J., Parker J.W. and Taylor C.R. (1982). Cytoplasmic as opposed to surface Ia antigens expressed on human peripheral blood lymphocytes and monocytes. *Clin. Exp. Immunol.* 49, 355-363.
- Hsu S-M, Raine L. and Fanger H. (1981). Use of the avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. *J. Histochem. Cytochem.* 29:577-580.
- Jannosy G., Tidman N., Selby W.S. Thomas J.A. Granger S., Kung P.C. and Goldstein G. (1980). Human T lymphocytes of inducer and suppressor type occupy different microenvironments. *Nature*, 288, 81-84.
- Knox J.M. Dodge B.G. and Freeman R.G. (1968). Erythema dyschromicum perstans. *Arch. Dermatol.* 97, 262, 272.
- Landay A., Gartland G.L. and Clement L.T. (1983). Characterization of a phenotypically distinct subpopulations of Leu-2+ cells that suppresses T cell proliferative responses. *J. Immunol.* 131, 2757-2761.
- Lerner A.B. (1959). Vitiligo. *J. Invest. Dermatol.* 32, 285-310.
- Ljunghall K., Loof L. and Forsum U. (1982). T lymphocyte subsets in the duodenal epithelium in dermatitis herpetiformis. *Acta Dermatol. Venereol. (Stockn)* 62, 485-489.
- Modlin R.L., Hofman F.M., Horwitz, D.A., Husmann, L.A., Gillis S., Taylor C.R. and Rea T.H. (1984). In situ identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin-2 and its receptor. *J. Immunol.* 132, 3085-3090.
- Moroshashi M., Hashimoto K., Goodman T.F. Jr., Newton D.E. and Rist T. (1977). Ultrastructural studies of vitiligo, Vogt-Koyanagi syndrome and incontinentia pigmenti achromians. *Arch. Dermatol.* 113, 755-766.
- Naughton G.K., Eisinger M. and Bystryk J.C. (1983). Detection of antibodies to melanocytes in vitiligo by specific immunoprecipitation. *J. Invest. Dermatol.* 81:540-542.
- Nordlund J.J. and Lerner A.B. (1979). Vitiligo: its relationship to systemic disease. In: *Dermatology Update*, Elsevier North Holland Inc., New York, pp 411-432.
- Nordlund J.J., Albert D. and Forget B. (1980). Halo nervi and the Vogt-Koyanagi-Harada syndrome. *Arch. Dermatol.* 116, 690-692.
- Pinkus H. (1959). Vitiligo-what is it? *J. Invest. Dermatol.* 32, 281-284.
- Ramirez C.O. (1957). Los cenicientos, problema clínico. Memoria del 1º Congreso Centroamericano de Dermatología, San Salvador, 5-8 de Diciembre de 1957.
- Ramirez C.O. (1966). Dermatitis cenicienta: Estudio epidemiológico de 139 casos. *Dermatol. Rev. Mex.* 10, 133-142.
- Riley. R.A. (1967). A study of the distribution of epidermal dendritic cells in pigmented and unpigmented skin. *J. Invest. Dermatol.* 48, 28-38.
- Rocha C., de Maubeuge J., Sarfati M., Song, M. and Delespesse G. (1984). Characterization of cellular infiltrates in skin lesions of atopic dermatitis by means of monoclonal antibodies. *Dermatologica* 169, 330-338.
- Soter N.A., Wand C. and Freeman R.G. (1969). Ultrastructural pathology of erythema dyschromicum perstans. *J. Invest. Dermatol.* 52, 155-161.
- Stevenson J.R. and Miura M. (1966). Erythema dyschromicum perstans (ashy dermatosis). *Arch. Dermatol.* 94, 196-199.
- Streilein J.W. (1983). Skin Associated Lymphoid Tissue. *J. Invest. Dermatol.* 80, 12s-16s

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