Original Research

The Immunopathology of Regression in Benign Lichenoid Keratosis, Keratoacanthoma and Halo Nevus

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ABSTRACT

BACKGROUND

Regression is a phenomenon present in a variety of cutaneous lesions. It is likely that similar immunologic mechanisms explain the phenomenon of spontaneous regression occurring in the various lesions.

METHODS

Twenty-seven specimens, nine each of halo nevus, keratoacanthoma, and benign lichenoid keratosis, including three examples each of predominantly early, mid, and late regression were examined with antibodies to HLA-II, CD1a, CD3, CD4, CD8, CD20, CD34, CD56, and CD68.

RESULTS

Epidermotropism of inflammatory cells, including CD1a positive, CD68 positive, CD3 positive, and CD8 positive cells, was present in benign lichenoid keratosis and keratoacanthoma, but not in halo nevus. In halo nevus, the nests of halo nevus cells tended to be infiltrated by CD1a positive, CD68 positive, CD3 positive, and CD8 positive cells. The blood vessels exhibited endothelial cell swelling with luminal narrowing and disruption within the dermis of all lesions. The CD1a positive cells were increased in number in lesional epidermis except in keratoacanthoma lesions where the density of CD1a positive cells was increased in the epithelial lip, but decreased within the epithelial portion of the keratoacanthoma proper. Conversely, the CD8 positive cells were scarce in the dermis below the epithelial lip of the keratoacanthoma, but increased in the dermis of the neoplastic epithelium. CD1a positive cells were also seen throughout the dermal portion of the lesion, particularly at the lesion base. In halo nevus, the CD1a positive cells and CD68 positive cells within the lesions were larger than those in non-lesional skin, indicating activation. The composition of the inflammatory infiltrate varied within each lesion type according to stage of regression, but T-lymphocytes predominated.

CONCLUSION

Cytotoxic T-cells may be the final common denominator of regression in benign lichenoid keratosis, keratoacanthoma, and halo nevus. In halo nevus, cytotoxic T-cells may play the predominant role in regression. In keratoacanthoma and benign lichenoid keratosis, cytotoxic T-cells play a pivotal role, but additional mechanisms may also be involved in the phenomenon of regression. Benign lichenoid keratoses progress through stages of regression accompanied by varying proportions of inflammatory cells, including CD3, CD4, and CD8 positive T-lymphocytes, natural killer cells, macrophages and Langerhans cells.

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INTRODUCTION

Spontaneous regression is a phenomenon present in a variety of cutaneous lesions that likely has an immunologic mechanism responsible for its pathogenesis. Recognition of antigens by the immune system is mediated by both a cell-mediated and a humoral-mediated response. T-cells interact with processed antigens via the T-cell receptor that requires the presence of major histocompatibility complex (MHC) molecules on the antigen-presenting cell. Processing of cellular proteins for presentation to CD4 positive helper T-cells is accomplished by antigen-presenting cells that bear MHC class II molecules. In turn, the activated CD4 positive helper T-cells may stimulate B-cells, via lymphokine production and release, to undergo differentiation with subsequent production of a specific antibody. Alternatively, the activated CD4 positive helper T-cells may activate CD8 positive cells (cytotoxic T-cells) that then participate in target cell destruction. Cytotoxic T-cells recognize foreign antigen in the context of MHC class I molecules and exert their cytotoxic effects either by fas-ligand dependent apoptosis or by a perforin-dependent mechanism of direct membrane damage. Damage to the target cell may then amplify the immune response via release of cytoplasmic antigens that are normally encrypted from the immune system. Once the foreign antigen is eliminated, the activated T-cells and antigen-specific B-cells undergo reduction in their cell numbers.¹

Halo nevi have been extensively studied as an *in vivo* model for the immunologic mechanism responsible for regression in melanoma.² The potential for spontaneous and complete regression is *sine qua non* in the definition of keratoacanthoma.³ Benign lichenoid keratosis has been postulated by some to be the inflammatory stage of regressing solar lentigines and reticulated seborrheic keratoses,^{4,5} while others suggest that it is a separate lesion.⁶ It is likely that these three lesions share a similar immunologic mechanism that explains the phenomenon of spontaneous regression occurring in each.

Halo nevus is a phenomenon involving several related conditions, including congenital melanocytic nevi,^{7,8} Spitz nevi,^{9,10} congenital giant nevocellular nevi,⁷ balloon cell nevi,¹¹ and atypical nevi¹² among others. Histologically, there is progressive degeneration of nevus cells surrounded by an inflammatory infiltrate. The involutional process has been divided into four stages, each characterized by the cellular profile of the inflammatory cell infiltrate. Stage I (pre-regression) is characterized by nests of nevus cells surrounded by a moderate number of T-lymphocytes. In early regression (stage II), the nests of nevus cells are in close contact with a greatly increased number of T-cells, and the number of both Langerhans cells and lysozyme-positive cells are also increased. Stage III (late regression) demonstrates isolated nevomelanocytes with mild atypia scattered among the inflammatory infiltrate. Finally, in stage IV (complete regression) no nevus cells are present, and

only a moderate number of inflammatory cells are present.¹³ Different phases of regression may coexist in the same nevus.

Keratoacanthoma shows hypertrophy of keratinocytes with cytoplasmic pallor and varying degrees of cytologic atypia, intraepithelial abscesses, transepidermal elimination of connective tissue, and varying degrees of immune response with patterns of regression.³ Three stages are seen in the evolution of the keratoacanthoma,¹⁴ somewhat analogous to the involutional process that takes place in halo nevi. In the early or immunostimulatory phase, a nodular or cup-shaped proliferation of mildly atypical cells with rare dyskeratotic cells¹⁵ is present, with columns of pale keratinocytes which invade the reticular dermis at a rate that either exceeds the capabilities of the immune response or betrays a defect thereof.¹⁴ The established lesion consists of larger, more irregular, infiltrating squamous nests and islands that have increased numbers of dyskeratotic cells and scattered mitotic figures¹⁵ abutting a reactive stroma containing dense infiltrates of lymphocytes and histiocytes with a variable admixture of eosinophils and plasma cells. Focal exocytosis of lymphocytes is present and contributes to the lichenoid pattern.¹⁴ In the regressing or desmoplastic stage, there is a superficial dermal lesion with scalloped epithelial remnants and dermal columns of neoplastic cells that are reduced in number within a matrix that may be cellular (early regression) or densely fibrous (late regression) with characteristics of a scar. Isolated nests of keratinocytes analogous to keratinous cysts and shallow craters with an irregular surface lined by cytologically bland keratinocytes may be present. Patterns of both partial regression and active invasive growth may coexist in the same lesion with the regressing component located centrally and the invasive component located at the lateral portion of the lesion.³

Benign lichenoid keratosis is a common lesion that some consider an inflammatory stage of regressing lesions such as solar lentigines or seborrheic keratoses,^{4,5} while others consider it a distinct lesion in and of itself.⁶ Benign lichenoid keratosis goes through stages of evolution or regression. Initially, there is a lichenoid inflammatory response accompanied by mild basal vacuolization and dyskeratotic keratinocytes. In more established lesions, basal vacuolization becomes more extensive with dyskeratotic keratinocytes also evident within the papillary dermis. In late stage lesions, marked basal vacuolization may progress to subepidermal clefting. There is also apoptosis as well as pigment within the dermal macrophages. At the periphery of these latter lesions, there are changes of regression with an increase in dermal vessels, a residual scant inflammatory infiltrate, and mild edema of the papillary dermis. There may be different phases of regression coexisting in the same lesion.

This project was undertaken to ascertain the events in the regression sequence of halo nevus, keratoacanthoma, and benign lichenoid keratosis. Knowledge and understanding of

these events will help to delineate the mechanism of the occurrence of regression in cutaneous lesions and explain its commonness.

MATERIALS AND METHODS

Nine specimens each of halo nevi, keratoacanthoma, and benign lichenoid keratosis were prospectively selected at the St. Joseph's Dermatopathology Laboratory in Houston, Texas. The nine specimens from each lesion type included three examples each of predominantly early, mid, and late regression stages. The stage I (pre-regression) halo nevus was defined by nests of nevus cells surrounded by a moderate number of T-lymphocytes. The stage II (early regression) halo nevus was defined by nests of nevus cells in close contact with a greatly increased number of T-cells with increased numbers of Langerhans cells and macrophages. The stage III (late regression) halo nevus was defined by isolated nevocmelanocytes with mild atypia scattered among the inflammatory infiltrate.¹³

Columns of pale keratinocytes invading the reticular dermis accompanied by a modest lymphohistiocytic inflammatory infiltrate defined the early stage of keratoacanthoma. The established lesion was defined by pale keratinocytes abutting a reactive stroma that contained a dense infiltrate of mononuclear cells in a lichenoid pattern. The desmoplastic stage was defined by reduced numbers of dermal columns of pale keratinocytes within a cellular matrix also containing isolated nests of keratinocytes and shallow craters with an irregular surface lined by cytologically bland keratinocytes accompanied by a moderate mononuclear inflammatory infiltrate.¹⁴

The benign lichenoid keratoses were assigned a stage based upon the amount of inflammation, degree of basal vacuolization, and presence of pigment within dermal macrophages. The early lesion contained a modest inflammatory infiltrate with mild basal vacuolization and few apoptotic keratinocytes. The stage II benign lichenoid keratosis contained a marked mononuclear inflammatory infiltrate with a moderate amount of basal vacuolization and

a modest amount of apoptotic keratinocytes. The late lesion contained a moderate mononuclear inflammatory infiltrate with marked basal vacuolization and apoptosis, as well as pigment within the dermal macrophages.

The specimens were fixed in 10% formalin and embedded in paraffin. Three micron sections of the specimens were cut and mounted on 3-aminopropyltrethoxy-silane-coated slides, dried, and deparaffinized prior to undergoing immunohistochemical staining with antibodies to HLA-II (Serotec clone WR18, Dudlington, Oxford, UK), CD1a (Ventana clone JPM30, Tucson, AZ, USA), CD3 (Ventana clone PS1), CD4 (Ventana clone 1F6), CD8 (Ventana clone 1A5), CD20 (Ventana clone L265), CD34 (Ventana clone QBEnd/10), CD56 (Ventana clone 1B6), and CD68 (Ventana clone KP-1). The staining was done according to the manufacturers' specifications using the avidin-biotin complex method. Nonimmune mouse sera was used as the negative control while positive controls consisted of appropriate tissue known to contain the antigens of interest. The signal was visualized with 3,3'-diaminobenzadine in chromogen solution which yielded a brown color at the site of the target antigen. The results were interpreted and quantified by routine light microscopy and evaluated on the basis of pattern and location of inflammatory cells. Quantification of the number of CD3, CD4, CD8, CD20, and CD56 positive inflammatory cells was done manually. The area of each lesion was measured with a micrometer. The absolute number of positively stained cells was calculated by dividing the number of positively stained cells by the area of the lesion. The average result for each lesion type was then calculated. The ratio of CD3 to CD20 positive cells and CD4 to CD8 positive cells was also calculated.

RESULTS

Epidermotropism of inflammatory cells, including CD1a positive, CD68 positive, CD3 positive, and CD8 positive cells, but not CD4 positive cells, was present in the benign lichenoid keratosis and keratoacanthoma lesions. Also, CD68 positive cells were present in the keratoacanthoma lesional epithelium but not in nonlesional epithelium. The

Figure 1. The nests of nevus cells in this halo nevus are infiltrated by CD8 positive cells.

Figure 2. CD34 positive blood vessels approximate the epidermis more closely within the lesion than in non-lesional skin in this benign lichenoid keratosis.

Figure 3. The density of CD1a positive cells is increased in the epithelial lip of this keratoacanthoma, but decreased within the epithelial portion of the keratoacanthoma proper.

Figure 4. In keratoacanthoma, CD20 positive cells are present in clusters at the base of the lesion.

Figure 5. A stage II benign lichenoid keratosis with a marked mononuclear infiltrate, a moderate amount of basal vacuolization, and a modest number of apoptotic keratinocytes.

halo nevus displayed compact orthokeratosis, but no epidermotropism of inflammatory cells. Rather, the nests of nevus cells tended to be infiltrated by CD1a positive, CD68 positive, CD3 positive, and CD8 positive cells (figure 1), but not CD4 positive cells. CD68 positive cells surrounded the nests in all stages of the halo nevus and penetrated the nests in the early and late regression lesions. In keratoacanthoma and benign lichenoid keratosis the blood vessels exhibited endothelial cell swelling with luminal narrowing and disruption within the dermis of the lesions. The CD34 positive blood vessels approximated the epidermis more closely within the lesion than in non-lesional skin and were increased in number at the base of both keratoacanthoma and benign lichenoid keratosis lesions (figure 2).

Proliferation of CD34 positive vessels in halo nevus was seen away from the nevus nests with some vessels approximating the epidermis, but not as closely as in the keratoacanthoma and benign lichenoid keratosis lesions. An increase in CD34 positive interstitial cells was present within the keratoacanthoma and benign lichenoid keratosis lesions. CD1a positive cells were increased in number in the epidermis of the benign lichenoid keratosis lesions, twice that of non-lesional epidermis, and were also seen throughout the dermal portion of the lesion, particularly at the lesion base. In the keratoacanthoma lesions, the density of CD1a positive cells was increased in the epithelial lip of the keratoacanthoma, but decreased within the epithelial portion of the keratoacanthoma proper (figure 3). Conversely, the CD8 positive cells were scarce in the dermis below the epithelial lip of the keratoacanthoma, but increased in the dermis of the neoplastic epithelium. In halo nevus, CD1a positive cells were increased in number in the overlying epidermis and follicles and were also present in areas of regression. The CD1a positive cells within the lesions were larger, 21 microns on average, than those in non-lesional skin, while the CD68 positive cells were also larger within the lesions. The number of CD56 positive cells in the early and late regression stages of halo nevus was increased over those seen in the benign lichenoid keratosis and keratoacanthoma lesions. In halo nevus, CD20 positive cells were present in clusters between nests of nevus cells, but did not infiltrate them, while in keratoacanthoma, CD20 positive cells were present in clusters at the base of the lesion (figure 4).

Histologically, the amount and composition of the inflammatory infiltrate in the benign lichenoid keratosis varied according to the stage of the lesion with the early lesion containing a modest number, the stage II lesion a marked number (figure 5), and the late lesion a moderate number of inflammatory cells. The early lesion contained primarily CD3 positive lymphocytes (CD3 to CD20 ratio 17:1) and was comprised of almost equal proportions of CD4 positive and CD8 positive lymphocytes (CD4 to CD8 ratio 1.6:1). The stage II lesion demonstrated a greater proportion of B-lymphocytes (CD3 to CD20 ratio 7:1). The

T-lymphocyte population was comprised of equal proportions of CD4 positive and CD8 positive lymphocytes (CD4 to CD8 ratio 1:1). The late lesion contained the greatest number of B-lymphocytes (CD3 to CD20 ratio 4:1), and the T-lymphocyte population was comprised of almost equal proportions of CD4 positive and CD8 positive lymphocytes (CD4 to CD8 ratio 0.9:1). In addition, the dermal inflammatory infiltrate also contained CD68 positive cells. The CD68 positive cells were also seen surrounding apoptotic keratinocytes in the epidermis of the early benign lichenoid keratosis. The HLA-II staining cells comprised the CD4 positive and CD68 positive cells in each lesion. CD56 positive cells, although few in number, were seen to increase from a rare cell in the early lesion (9 cells/mm²) to scant in the stage II lesion (23 cells/mm²) and were most numerous in the dermal inflammatory infiltrate in the late lesion (31 cells/mm²) (tables 1 and 2).

Histologically, the amount of the lymphohistiocytic inflammatory infiltrate in the keratoacanthoma lesions varied according to the stage of the lesion. The early lesion contained a modest inflammatory infiltrate located at the interface of the advancing tumor and the reticular dermis. The established lesion contained a dense infiltrate of mononuclear cells at the interface between columns of lesional keratinocytes and the dermis (figure 6). The late lesion contained a moderate mononuclear inflammatory infiltrate located both at the advancing margin of the tumor as well as within the reactive stroma. The composition of the inflammatory infiltrate in the keratoacanthoma lesions varied with lesion stage. The early lesion was comprised of

primarily CD3 positive lymphocytes (CD3 to CD20 ratio 14:1) with CD20 positive lymphocytes located in clusters concentrated just below the dermal-epidermal junction. The T-lymphocyte population was mostly composed of CD4 positive lymphocytes (CD4 to CD8 ratio 5:1). The established lesion demonstrated a greater proportion of B-lymphocytes (CD3 to CD20 ratio 8:1) with CD20 positive lymphocytes located primarily in groups at the base of the lesion. The T-lymphocyte population was comprised of an increased number of CD8 positive lymphocytes over that of the early lesion (CD4 to CD8 ratio 3:1). The late keratoacanthoma contained the greatest number of B-lymphocytes (CD3 to CD20 ratio 4:1) and the T-lymphocyte population was comprised of almost equal proportions of CD4 positive and CD8 positive lymphocytes (CD4 to CD8 ratio 1:1). The lesions also contained CD68 positive mononuclear cells. In the early lesion, the CD68 positive cells were concentrated at the lesion periphery with very few within the substance of the tumor. The established lesion showed CD68 positive cells concentrated at the periphery of the tumor, while the late lesion showed the CD68 positive cells concentrated at the periphery with few within the fibrotic area at the base of the tumor. The HLA-II staining cells comprised the CD68 positive and CD4 positive cells in each lesion. Although CD56 positive cells were rare in all stages of keratoacanthoma, they did increase with the stage of regression, being least in the early lesion (9 cells/mm²), scant in the stage II lesion (16 cells/mm²), and most numerous in the dermal inflammatory infiltrate of the late lesion (21 cells/mm²) (tables 1 and 2).

Table 1. Quantification of the inflammatory cell infiltrate reflecting the absolute number of cells per area.

	Number of positive cells/mm ²				
	CD20	CD3	CD4	CD8	CD56
Early KA	48	665	553	107	9
Mid KA	67	249	181	67	16
Late KA	208	1023	511	452	21
Early BLK	51	878	542	416	9
Mid BLK	173	1260	643	625	23
Late BLK	297	1267	632	642	31
Early HN	200	2047	1058	1003	10
Mid HN	174	1992	1051	1037	42
Late HN	97	1551	712	840	46

KA, keratoacanthoma; BLK, benign lichenoid keratosis; HN, halo nevus.

Table 2. Ratios of the inflammatory cell infiltrate.

	CD3:CD20	CD4:CD8
Early KA	14:1	5:1
Mid KA	8:1	3:1
Late KA	5:1	1:1
Early BLK	17:1	2:1
Mid BLK	7:1	1:1
Late BLK	4:1	0.9:1
Early HN	10:1	1:1
Mid HN	11:1	1:1
Late HN	16:1	0.9:1

KA, keratoacanthoma; BLK, benign lichenoid keratosis; HN, halo nevus.

Figure 6. The predominantly established keratoacanthoma containing pale keratinocytes abutting a reactive stroma with a dense infiltrate of mononuclear cells arranged in a lichenoid pattern.

Histologically, the pre-regression stage of halo nevus showed nests of nevus cells surrounded by a moderate number of T-lymphocytes. In early regression, the number of T-cells greatly increased with a concomitant increase in Langerhans cells (figure 7). Late regression lesions demonstrated isolated scattered nevus cells among the moderate inflammatory infiltrate, with equal numbers of CD4 and CD8 positive cells within the regressed areas. The composition of the mononuclear inflammatory infiltrate varied with the stage of the halo nevus. The pre-regression halo nevus was comprised primarily of CD3 positive lymphocytes (CD3 to CD20 ratio 10:1) that were present both within and between the nests. CD20 positive lymphocytes were distributed both singly and in groups throughout the lesion, but not within the nevus nests. The T-lymphocyte population was comprised of an almost equal number of CD4 and CD8 positive lymphocytes (CD4 to CD8 ratio 1:1). The early regression halo nevus demonstrated the greatest proportion of B-lymphocytes (CD3 to CD20 ratio 11:1) present between nests of nevus cells. The T-lymphocyte population, which was present between and within nests of nevus cells, was comprised of an equal number of CD4 and CD8 positive lymphocytes (CD4 to CD8 ratio 1:1). The late regression lesion contained the least number of B-lymphocytes (CD3 to CD20 ratio 16:1), and the T-lymphocyte population was comprised of an almost equal proportion of CD4 positive and CD8 positive lymphocytes (CD4 to CD8 ratio 0.9:1) and was present between and within nests of nevus cells. The HLA-II staining cells were comprised of the CD68 positive and CD4 positive cells in each lesion (tables 1 and 2).

DISCUSSION

It is likely that halo nevus, keratoacanthoma, and benign lichenoid keratosis share a common immunologic mechanism that explains the phenomenon of regression. Cell-mediated immunity is likely the primary event in regression. However, humoral immunity may also play a role. The majority of the inflammatory cell infiltrate is

Figure 7. In predominantly early regression (stage II) halo nevus, nests of nevus cells are surrounded by a greatly increased number of T-cells accompanied by an increased number of Langerhans cells and macrophages.

derived from T-cell lineage, either cytotoxic or suppressor cells showing signs of activation, or CD4-positive helper T-cells.

T-cells, particularly CD8 positive cytotoxic T-cells, appear to be the key effector cell of regression in all three types of lesions. In halo nevus, most T-cells which infiltrate the tumor nests have been selected out of the circulating T-cell population due to the expression of cutaneous lymphocyte associated antigen, a protein involved in skin homing.¹⁶ The T-cells undergo activation and oligoclonal proliferation, likely as the result of the specific recognition process of a small number of tumor antigens.¹⁷ The activated T-cells express CD69 that is a very early activation marker, and this suggests that activation of the T-cells occurs *in vivo*.¹⁸ The nature of the tumor antigens in halo nevus is unknown. However, they may be autoantigens to which the immune system is normally tolerant. Thus, the regression occurring in halo nevus may represent lysis of a distinctive cell type through an autoimmune process mediated by an oligoclonal T-cell response.¹⁹ Melanocytes strongly express MHC class I molecules¹⁹ that allow them to be targeted and destroyed by cytotoxic T-cells. The predominance of CD8 positive cells, particularly in the late regression stage of halo nevus, suggests the importance of the cytotoxic T-cell as the effector cell in regression.

In contrast to the expression of cutaneous lymphocyte antigen in halo nevus, cutaneous lymphocyte antigen expression is not evident in benign lichenoid keratosis.⁶ Thus, it has been suggested that the infiltrating lymphocytes in benign lichenoid keratosis might be bystander T-cells and that the lesions of benign lichenoid keratosis might be caused by nonspecific infiltrates.⁶ In the current study, the population of T-cells comprised the majority of the inflammatory cell infiltrate, the composition of which varied between the stages of regression. The increase of CD68 positive and CD56 positive cells from early to late regression combined with an increase in cytotoxic T-cells and B-cells suggests that these cells act in concert to achieve

regression with the cytotoxic T-cells playing a key role. The epidermotropism of CD1a, CD68, CD3, and CD8 positive inflammatory cells in the lesion itself is evidence of the effector cells of regression targeting the lesional cells. Initial studies of keratoacanthoma showed no role for immunity in regression. Some found no increase in CD8 positive cells in keratoacanthoma and thus postulated that the CD4 positive cells are important mediators of regression of keratoacanthoma. In the current study, CD4 positive T-cells comprised the majority of the CD3 T-cell positive population in the early and mid regression stages. However, CD8 positive T-cells comprised the majority of the CD3 positive T-cell population in the late stage of regression. This suggests that although CD4 positive T-cells may play an early role in regression of keratoacanthoma lesions through augmentation of CD8 positive T-cell cytotoxicity, B-cell proliferation, and natural killer (NK) cell cytotoxicity via cytokine secretion,¹⁹ cytotoxic T-cells appear to be the key effector cell in late regression. The epidermotropism of CD1a, CD68, CD3, and CD8 positive inflammatory cells in the lesion, and not CD4 positive cells, is evidence for this theory.

In the current study, the proportion of CD8 positive cells increased at the expense of CD4 positive cells in all lesion types from early to late regression, except in halo nevus where the populations of CD4 positive and CD8 positive T-cells were present in almost equal numbers in all stages of regression. It is intuitive that cytotoxic T-cells would increase with lesion destruction. These results agree with the literature regarding CD4 positive and CD8 positive cutaneous T-lymphocyte subsets present in inflammatory dermatoses in which CD4 positive lymphocytes predominate over CD8 positive lymphocytes.^{20,21}

The role of B-cells in the phenomenon of regression is not known. Some suggest that the paucity of B-cells within the inflammatory infiltrate in halo nevus suggests that they do not have a significant role in regression.¹³ Others suggest that humoral immunity may play a role in regression of halo nevus. Patients with halo nevi produce antibodies against the cytoplasm of melanoma cells that disappear with regression or surgical excision.²² This antibody production likely occurs after cell-mediated lysis of the nevus cells with concomitant release of the nevocellular antigen responsible for B-cell activation.²³ Alternatively, activated CD4 positive helper T-cells may stimulate B-cells via lymphokine production and release to undergo differentiation with subsequent production of a specific antibody.¹ The increase in B-cells with increasing stage of regression in benign lichenoid keratosis and keratoacanthoma suggests that they do play a role in regression of these lesions, perhaps by producing specific antibodies to the autoantigen involved in tumor cell lysis. The fact that the B-cells do not infiltrate the nests in halo nevus or the epidermis in benign lichenoid keratosis or keratoacanthoma supports this theory.

CD68 positive tumor-infiltrating macrophages were observed in all lesions, but were not present in perilesional

skin. It is possible that these macrophages take part in mediating regression. The amount of CD68 positive cells present may increase with activation and maturation of macrophages, such as occurs in inflammation and neoplasia.²⁴ The CD68 positive macrophages were larger in the halo nevus, suggesting an active role in regression of halo nevus.

NK cells play a role in host defense and may mediate tumor surveillance.^{25,26} They target transformed cells that have down-regulated or lost the expression of MHC class I molecules while sparing normal cells that express adequate amounts of MHC class I molecules.^{25,27} The MHC class I related molecules are activators of both NK cells and T-cells via interaction with NKG2D.²⁸ NK cells also secrete cytokines, including interferon- γ , granulocyte-macrophage colony stimulating factor, and tumor necrosis factor- α that can modulate the immune response.²⁹ T-cell activation is accomplished through simultaneous engagement of the T-cell receptor and a costimulatory receptor resulting in triggering of T-cell effector function, proliferation, and survival.³⁰ T-cells may use the lectin-like NKG2D receptor for costimulation.³¹ NKG2D is involved in the natural killing of some tumor targets,²⁶ particularly those of epithelial derivation.³¹ Natural cytotoxicity receptors play a major role in the lysis of most tumor cells. This is underscored by the close correlation between the surface density of natural cytotoxicity receptors and the magnitude of the NK-mediated cytotoxicity. Indeed, only those cells expressing bright natural cytotoxicity receptor expression by flow cytometry efficiently kill most tumor cell lines.³²

In the current study, the number of CD56 positive NK cells varied in each of the three lesion types, as well as with each of the stages of regression. They progressively increased in all lesions from the early through the late stages. They were present in the greatest amount within the halo nevi. There are two possible explanations for the paucity of CD56 positive NK cells in the current study. The first is that widely used NK cell markers including CD16 and CD56 are not very sensitive for NK cells in paraffin-embedded tissue.^{33,34} Thus, more NK cells could have been present that did not stain with CD56. Alternatively, there is the possibility that only a small number of NK cells must be present to stimulate T-cell clonal expansion and function. This latter explanation is supported by the absolute increase in CD56 positive cells with stage of regression in the lesions studied. NK cells share some characteristics with cutaneous T-lymphocytes, including expression of CD8 although at a lower surface density.³⁴ Indeed, a so-called NK-like activity exists, mediated by a subset of activated CD8 positive cutaneous T-lymphocytes, and refers to the ability of these cells to lyse various NK-susceptible tumor target cells.³⁴

CD1a positive Langerhans cells were found to be increased in the epidermis and at the lesion periphery in all stages examined of benign lichenoid keratosis, keratoacanthoma, and halo nevus. Langerhans cells are stellate, mobile,

immature dendritic cells that extend their delicate processes in many directions from the cell body and are located above the basal layer of proliferating keratinocytes. They play an important role in the cell-mediated immune response and stimulate T-cells via surface expression of both MHC class I and II antigens.¹³ When they encounter a powerful immunologic stimulus, dendritic cells mature and move into the dermal lymphatics in search of antigen-specific T-cells. T-cells need the antigen processed and presented to them by antigen-presenting cells. The T-cell antigen receptor recognizes fragments of antigens bound to molecules of the MHC on the surface of the antigen-presenting cells. In order to generate cytotoxic killer cells with the ability to eliminate tumor cells, dendritic cells present antigenic peptides complexed to MHC class I molecules that are recognized by CD8 positive T-cells. Once activated, the CD8 positive T-cells can directly kill a target cell. Dendritic cells activate and expand the T-helper cell population, which then induces B-cell growth and antibody production.³⁵ Extracellular antigens that have entered the cell are presented by MHC class II molecules to T-helper cells which, when stimulated, have profound immunoregulatory effects. The presence of increased dendritic cells in all stages of the lesions examined highlights the importance of dendritic cells in control of the immune response and suggests their importance in the phenomenon of regression. In halo nevus, Langerhans cells may act in concert with the melanocytes to stimulate T-cell mediated nevus cell lysis.¹ Also, the Langerhans cells in halo nevus were larger, suggesting an active role for Langerhans cells in the regression of halo nevus.

In the keratoacanthomas, there were more CD1a positive cells in the epithelial lip than in the keratoacanthoma proper, while CD8 positive cells were scarce in the dermis below the epithelial lip, but increased in the dermis of the neoplastic epithelium. This is likely due to the functions of each of the cells in that Langerhans cells activate cytotoxic T-cells that then target and destroy the neoplastic cells. Thus, the presence of more cytotoxic T-cells in the center of the lesion coincides with a later phase of regression, perhaps in an older part of the lesion, while the presence of increased Langerhans cells at the periphery or epithelial lip coincides with an earlier phase of regression, perhaps in an infiltrating portion of the lesion.

In conclusion, cytotoxic T-cells may be the final common denominator of regression in halo nevus, keratoacanthoma, and benign lichenoid keratosis in that their presence is associated with lysis of tumor cells. In the lesions of keratoacanthoma, CD4 positive cells may be pivotal in the early and mid stages of regression. However, it is the CD8 positive cytotoxic T-cells that appear to play a more dominant role in the late stage of regression. The greater percentage of CD8 positive cells in halo nevus may be due to the combined effect of Langerhans cells and melanocyte stimulation of cytotoxic T-cells, while this is not the case in keratoacanthoma and benign lichenoid keratosis. The

increase in absolute number of NK cells with progressive stage of regression suggests that although tumor immunity mediated by NK cells may not be the main mechanism involved in regression, NK cells may play an ancillary role in T-cell activation and modulation of the immune response. Benign lichenoid keratosis lesions progress through stages of regression in a similar manner to those of halo nevus and keratoacanthoma lesions and are accompanied by varying proportions of inflammatory cells, including CD3 positive, CD4 positive, CD8 positive, and CD20 positive lymphocytes, NK cells, macrophages, and Langerhans cells. In all three types of lesions, an immunologically competent epidermis seems to be required to initiate or participate in the regressive process in conjunction with the vascular, stromal, and cellular infiltrates.

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