0022-202X/80/7505-0388$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 75:388–393, 1980

Vol. 75, No. 5
Printed in U.S.A.

Melanoma. An Ultrastructural Study of the Host Inflammatory and Vascular Responses

ANN M. DVORAK, M.D., MARTIN C. MIHM, JR., M.D., JUSTINE E. OSAGE, B.A., AND HAROLD F. DVORAK, M.D.

From the Departments of Pathology, Beth Israel Hospital, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Electron microscopic studies of 4 surgically excised human primary superficial spreading melanomas of the skin were done with special emphasis on the host inflammatory and vascular responses to tumor.

Host cells participating in the inflammatory response included numerous small lymphocytes, activated macrophages, and mast cells. Some mast cells had lost local portions of granule content, but complete degranulation of mast cells was not seen. Activated macrophages had avidly ingested melanin granules and cellular debris and often were close to dying tumor cells. Lymphocytes, many displaying motile forms, were actively invading the epidermis; formed perivascular cuffs around damaged vessels; and were in intimate contact with living, damaged, and dead melanoma cells. Basophils, eosinophils, and neutrophils were absent from inflammatory infiltrates. Changes of the microvasculature included focal endothelial necrosis and hypertrophy as well as basal lamina changes indicative of repeated episodes of endothelial injury, necrosis and regeneration.

These findings are discussed in relationship to a number of recent studies of the immunologically-mediated inflammatory responses to contact allergy, graft rejection, and syngeneic tumor rejection, in man and animals.

Recent studies, using improved light microscopic techniques, as well as ultrastructural and immunological approaches, of a variety of cell-mediated immunologic inflammatory reactions in man and animals, have revealed a number of new findings related to the host responses to a variety of biologically important foreign materials [1]. Thus, we have identified inflammatory cells, sequenced their arrival and described some of their functional characteristics [2,3]. We have also defined a number of microvascular changes [4] and have noted that the induration so characteristic of many of these lesions is produced by fibrin in the interstitial spaces. These studies include detailed studies of contact allergy in man [2,3]; graft rejection in man [5]; and syngeneic tumor rejection in guinea pigs [6–8]. Because of the striking findings related to basophilic leukocyte infiltration and degranulation; mast cell degranulation and replication; vascular necrosis, hypertrophy, replication and basal lamina changes; and fibrin formation, we undertook these studies in an attempt to relate some or all of these findings to the host response to tumors in man.

MATERIALS AND METHODS

Human primary melanomas, classified grossly and microscopically as superficial spreading type according to the classification of Clark et al [9], were fixed for electron microscopy at 20° by immersion of small blocks as previously described [4].

Tissue blocks were then washed overnight in 0.1 M sodium cacodylate buffer, pH 7.4, 4°C, and postfixed for 2 hr in 1.5% collidine-buffered osmium tetroxide at 20°C. Preceding dehydration in a graded series of alcohols, the specimens were washed 3 times in 0.05 sodium maleate buffer, pH 5.2, stained en bloc for 2 hr with uranyl acetate (2%), and washed 3 times in 0.05 M sodium maleate buffer, pH 5.2 [4]. Tissues were infiltrated with constant agitation and embedded in a low viscosity media as described by Spurr [10]. One micron alkaline Geimsa-stained Spurr-embedded sections [11] were examined by light microscopy and areas selected for study by electron microscopy included those with active inflammation, desmoplasia, and changes involving the microvasculature. Thin sections were cut with a diamond knife, stained with lead citrate and multiple blocks were examined in a Philip 301 electron microscope from a total of 4 melanomas.

RESULTS

Inflammatory cells consisted of small lymphocytes (Fig 1,4–6, 8, 9) and macrophages (Fig 7). Granulocytes were not found in this study. Macrophages were often filled with membrane-bound phagosomes packed with heavily pigmented melanin granules. Cellular debris was also present in macrophage phagolysosomes. Small lymphocytes, some showing motile configurations, (Fig 4) were located in the epidermis and dermis. They formed close contacts with viable (Fig 1, 4) and dead tumor cells (Fig 5, 6, 8) often in a rosette formation of a number of small lymphocytes surrounding one large tumor cell (Fig 8). Small lymphocytes formed prominent perivenular cuffs around vessels in the dermis which showed the full range of changes described below.

Mast cells were numerous and predominantly perivascular in location. These markedly elongated cells were primarily fully granulated and contained numerous cytoplasmic filaments (Fig 9). Complete loss of all granules or focal loss of single granules was not observed. Rather, some cells showed irregular areas of lucency within individual granules which may represent partial *in vivo* granule content losses (Fig 10).

Tumor cells varied from easily identifiable melanin granule containing cells (Fig 1, 2, 4) to cells displaying no mature or immature granules (Fig 3, 5–8). Cell size and cytoplasmic and nuclear details easily allowed identification of these cells. Tumor cell injury was characterized by accumulations of focal nonmembrane-bound, moderately-dense cytoplasmic lipid droplets (Fig 2), nuclear pyknosis (Fig 5), and extreme cytoplasmic lucency and swelling (Fig 7–8). These changes were often seen in cells surrounded by lymphocytes (Fig 8).

Vascular pathology was extensive. No fibrin or platelet intravascular material was seen. Small venules showed focal endothelial cell necrosis (Fig 11) and hypertrophy (Fig 12*B*, 13). Necrosis was characterized by lucency and cell swelling with some membrane disruption (Fig 11). Hypertrophy of endothelial cells was extensive and often reduced the vascular lumen to a slitlike opening (Fig 12*B*). Such enlarged endothelial cells with contracted nuclei showed a marked increase in Weibel-Palade bodies and cytoplasmic filaments (Fig 13). Some endothelial cells had numerous elongated surface villous processes. Basal lamina increases were apparent and diverse. Many vessels showed concentric multilamellar lamina densa structures (Fig 12*B*, 13), whereas others showed a marked uniform thickening of lamina densa (Fig 12*A*). Cellular debris was trapped within these layers. Extensive increases in collagen fibers interspersed within the basal lamina area and subjacent to it were also seen.

Manuscript received December 4, 1979; accepted for publication May 22, 1980.

This work was supported in part by USPHS grant CA-19141.

Reprint requests to: Dr. Ann Dvorak, Department of Pathology, Beth Israel Hospital, Boston, MA 02114.

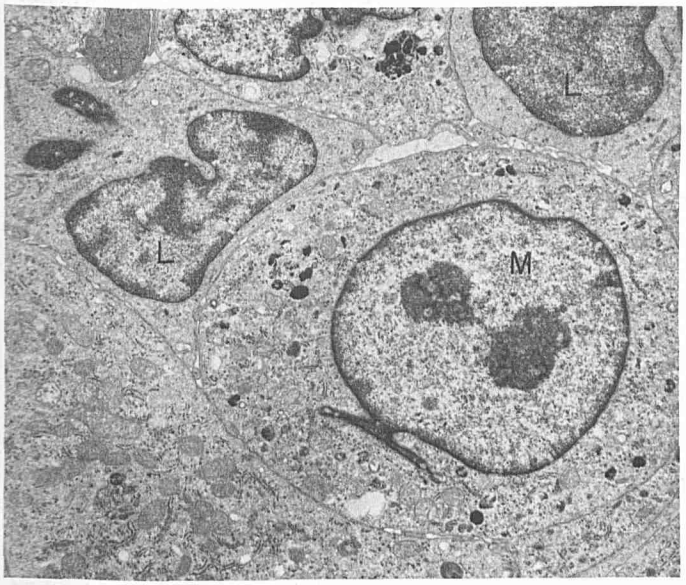

FIG 1. Large, viable melanoma cell (*M*) in the epidermis with prominent nucleoli and cytoplasmic melanin is contacted by 2 lymphocytes (*L*) (reduced from ×8000).

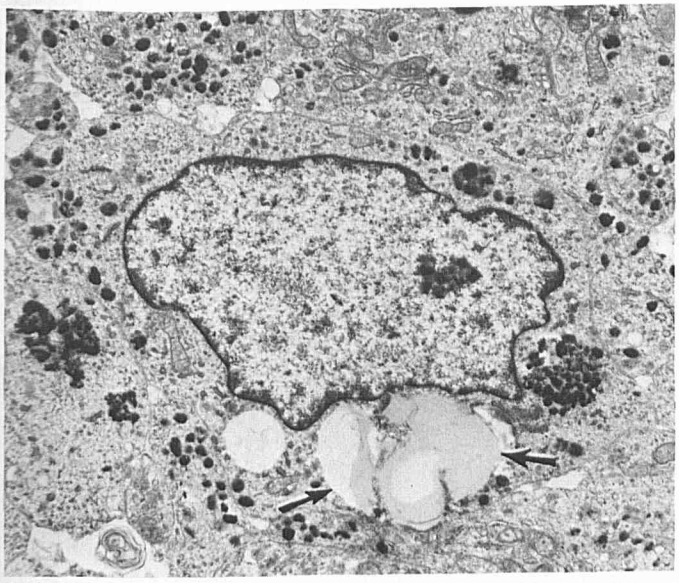

FIG 2. Viable melanoma cell in the epidermis with several large intracytoplasmic lipid accumulations (*arrows*) (reduced from ×8500).

DISCUSSION

In these electron microscopic studies of primary human superficial spreading malignant melanomas of the skin, we have described some host inflammatory and microvascular responses to tumor. The inflammatory cells predominantly present were small, round and motile lymphocytes, and activated macrophages. Granulocytes and plasma cells were absent. Tissue mast cells were prominent and some showed focal losses of the contents of individual granules. Tumor cells tightly contacted by a single lymphocyte or completely surrounded by as many as 6 lymphocytes were viable, damaged, and dead. Macrophages were avidly phagocytic for the resultant cellular debris. Microvascular changes, primarily affecting lymphocyte-cuffed venules, included focal endothelial cell necrosis, hypertrophy, and basal lamina changes.

The dying and dead tumor cells seen in these melanomas were usually in contact with lymphocytes suggesting that some tumor killing effector cells in these cases were lymphocytes.

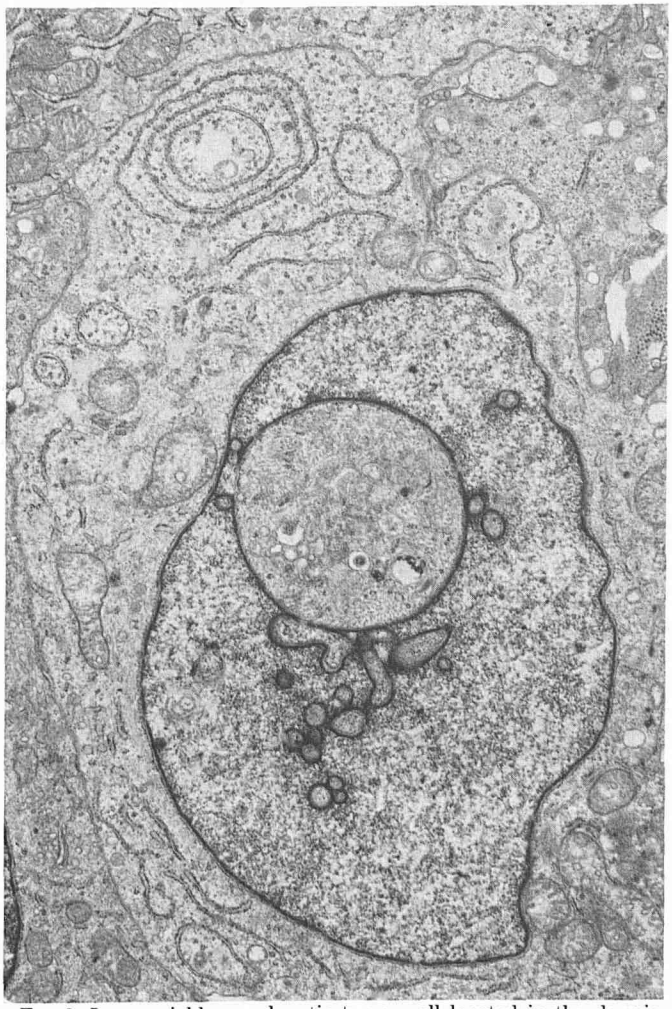

FIG 3. Large, viable, amelanotic tumor cell located in the dermis shows a large nuclear cytoplasmic invagination (reduced from ×11,500).

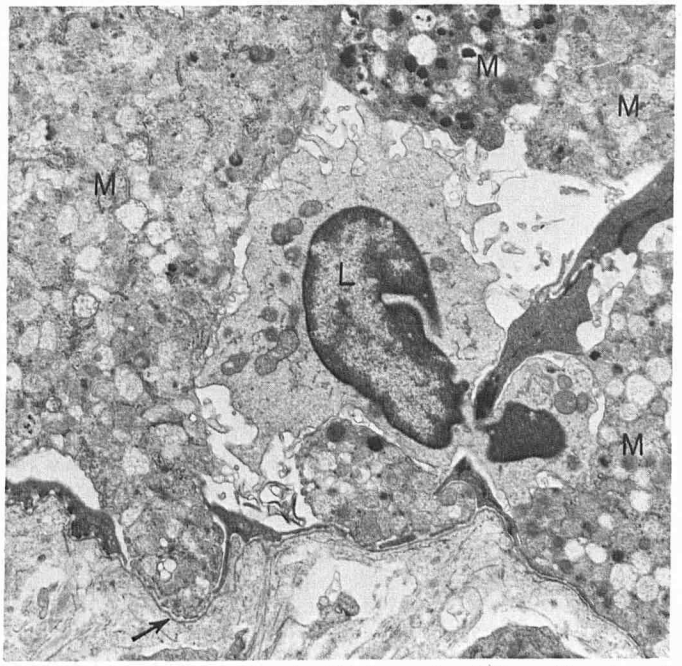

FIG 4. A motile lymphocyte (*L*) has entered the epidermis and contacted surrounding melanoma cells (*M*). One tumor cell process is pushing the epidermal basement membrane into the papillary dermis (*arrow*) (reduced from ×8000).

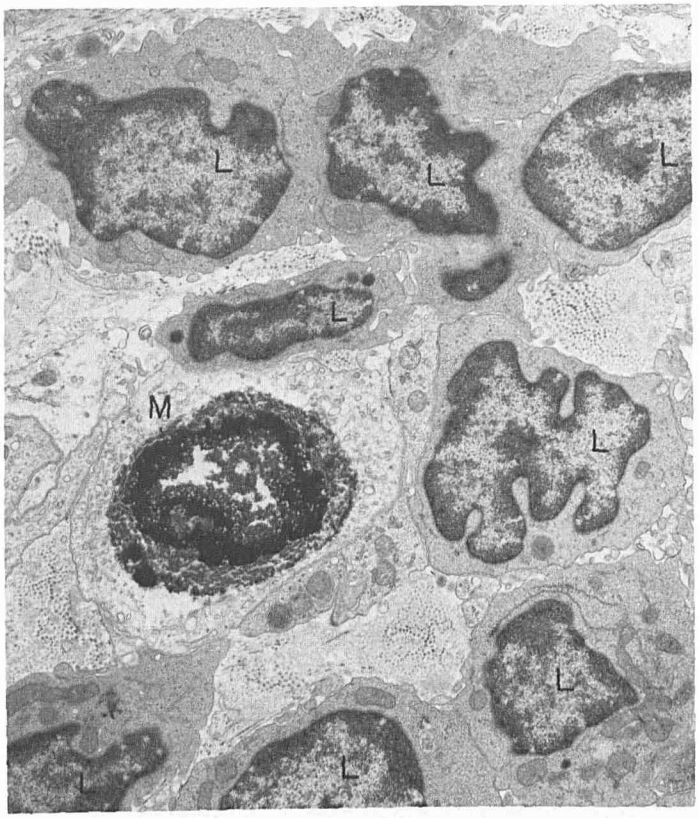

FIG 5. One dead tumor cell (*M*) with nuclear pyknosis is surrounded and contacted by a large number of small lymphocytes (*L*) (reduced from ×9500).

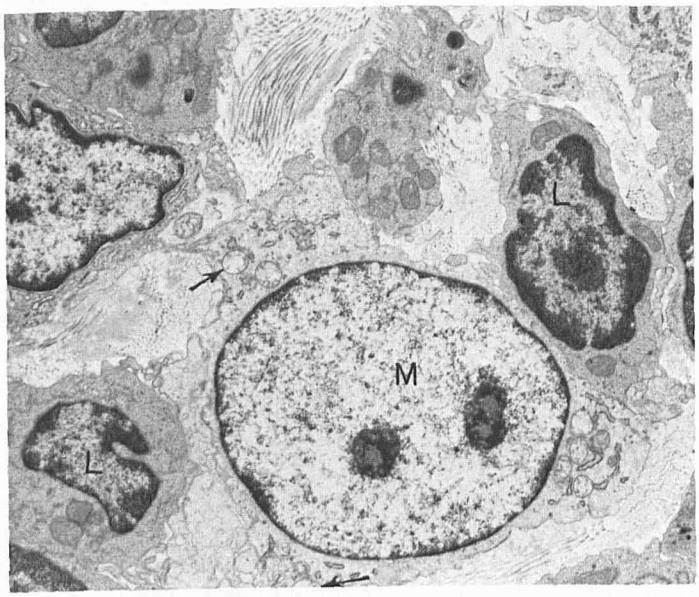

FIG 6. A large amelanotic tumor cell (*M*) is contacted by 2 tightly adherent lymphocytes (*L*). Mitochondrial swelling (*arrows*) may reflect lymphocyte-mediated cell injury (reduced from ×9000).

Lymphocytes are capable of killing tumor cells, as studied in a large number of *in vitro* systems [12]. The killing mechanism in some instances requires lymphocyte-tumor target cell contact [13,14]. Lymphocytes may also release lymphotoxin which is cytotoxic to many cells, as well as to tumor cells [15–17]. Other cells associated with tumor destruction include macrophages [18], polymorphonuclear neutrophils [19], and basophilic leukocytes [6,20]. The latter 2 were not present in these tumors. The macrophages present were not engaged in piece-meal phagocytosis or extrusion of lysosomes into tumor cells, as has been described by others [21,22].

More recently, extensive morphologic, immunologic, and biochemical studies of syngeneic tumor rejection in guinea pigs have revealed an important mechanism for tumor destruction which depends, in part, on the microvasculature [7,8]. In these studies, individual tumor cells contacted by lymphocytes at the periphery of tumors were killed, much as we have seen in the

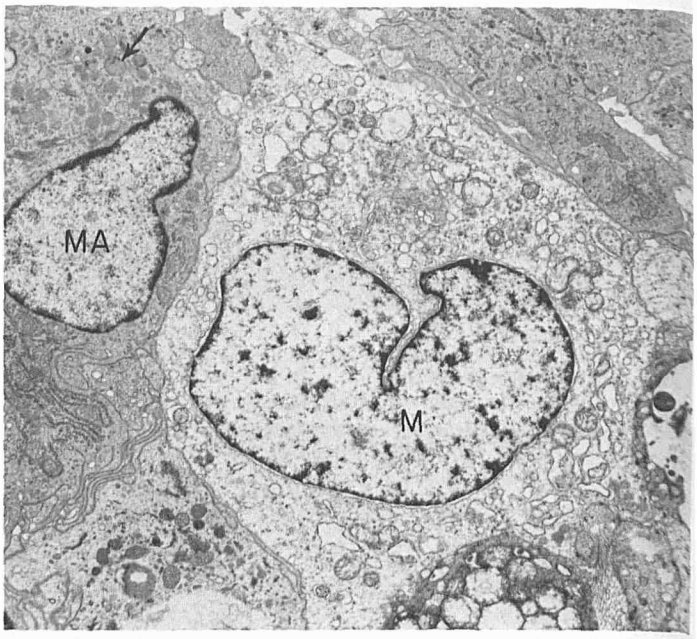

FIG 7. Tumor cell (*M*) in contact with a lysosome-containing (*arrow*) macrophage (*MA*) shows many signs of cell injury such as perinuclear cisternal blebs and numerous swollen cytoplasmic organelles (reduced from ×7000).

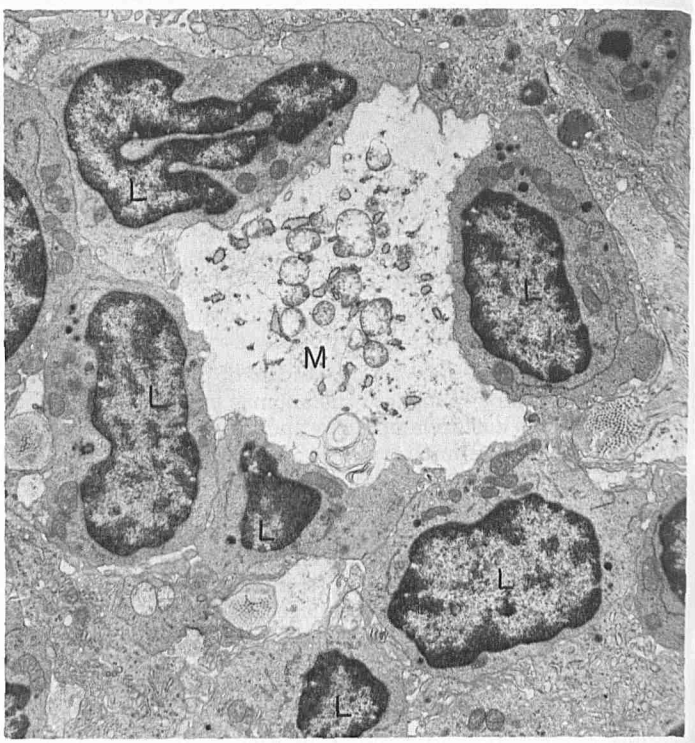

FIG 8. Completely lucent, dead tumor cell (*M*) contacted tightly by a rosette of lymphocytes (*L*) (reduced from ×7500).

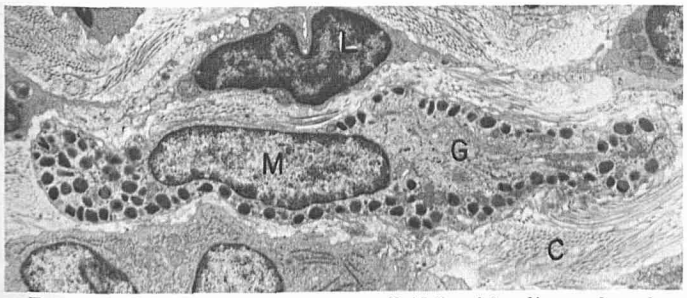

FIG 9. This elongated young mast cell (*M*), with adjacent lymphocyte (*L*), has a complete complement of granules, Golgi area prominence, and is enmeshed in dense collagen (*C*) (reduced from ×6000).

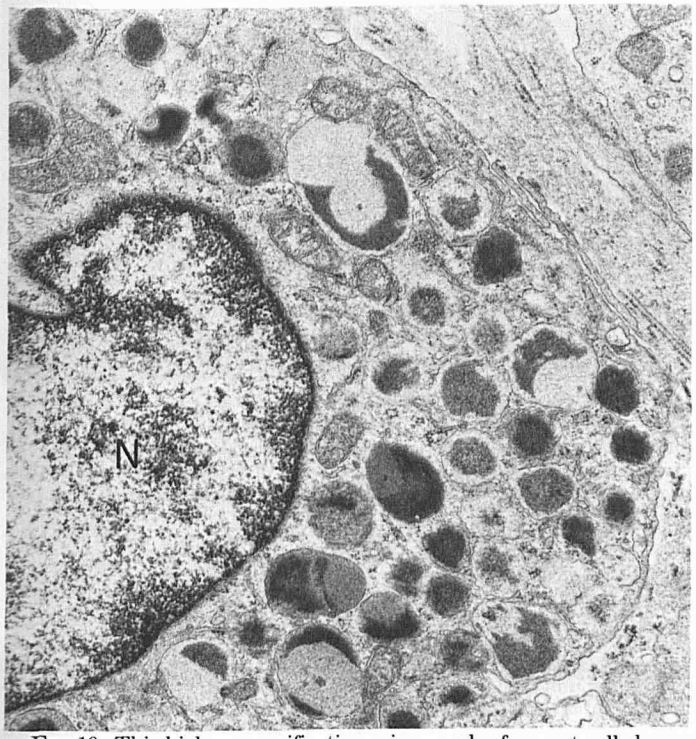

FIG 10. This higher magnification micrograph of a mast cell shows focal, irregular losses of dense content from individual granules (N—nucleus) (reduced from ×18,000).

human primary malignant melanomas studied here. Of great interest was the focal and sudden necrosis of large islands of cells at times when the tumor mass was so small that the phenomenon could not be explained by an overgrowth of blood supply by tumor. This sudden and focal infarction of tumor was associated ultrastructurally with numerous nonmembrane-bound lipid accumulations in damaged tumor cells, quite similar to the changes present in this study. Moreover, we have seen similar changes in tumor cells of halo nevi in the depigmented areas of tumor regression (AMD, unpublished data).

As an explanation for the focal infarctions of tumor described above, we examined, in the guinea pig model, the microvasculature and found extensive endothelial cell necrosis [8]. These damaged vessels could be responsible, ultimately, for the death of tumor. The findings reported, in the guinea pig model and in human melanomas, of endothelial cell necrosis, hypertrophy, and replicating basal lamina are diagnostic of repeated focal injury to the microvasculation as was demonstrated by the studies of Vracko and Benditt [23,24]. The mechanism of such injury is unknown but is not specific for tumors. We have found similar changes in a large study of such cell-mediated phenomena as contact allergy and graft rejection in humans [4,5]. The prominent perivascular cuffs of lymphocytes in all of these lesions suggest the possibility that lymphokines produced by these lymphocytes may be responsible for the observed effects of the microvasculature. The mechanism of such an effect does not seem to require cell contacts since lymphocytes were nearly always located below the basement membranes of the vessels and were not in contact with acutely damaged endothelia.

Our demonstration of the vessel changes described above in skin graft rejection in man was dramatic and was associated with the acute devitalization (infarction) of portions of the graft [5]. The mechanism is therefore quite similar to that which we have proposed for syngeneic tumor rejection in the guinea pig models.

Others have reported basal lamina changes similar to those reported here in nonspecific injury to skeletal muscle, neoplasia of epithelial or endothelial cells or pericytes, diabetes, collagen diseases, certain forms of arteriosclerosis, hypertension, a variety of neuropathies, and kidney and lung allograft rejection [1]. Therefore, while these basal lamina changes are indeed characteristic features of cell-mediated immunity, they are by no means specific for this form of immunologic response. The pathogenesis of basal lamina redupliction in cell-mediated immunity, as in other pathologic situations, is probably related to changes affecting the endothelial cells [23,24].

To summarize, in our studies of human melanomas we have seen 2 possible mechanisms for the death of tumor: (1) individual tumor cell killing by lymphocytes, and (2) acute and chronic damage to vessels which may result in ischemia and local tumor damage of varying amounts. Both of these mechanisms were also seen in our studies of syngeneic tumor rejection in guinea pigs [6–8]. In this model, the vessel necrosis was clearly shown to be associated with local infarction of islands of tumor cells.

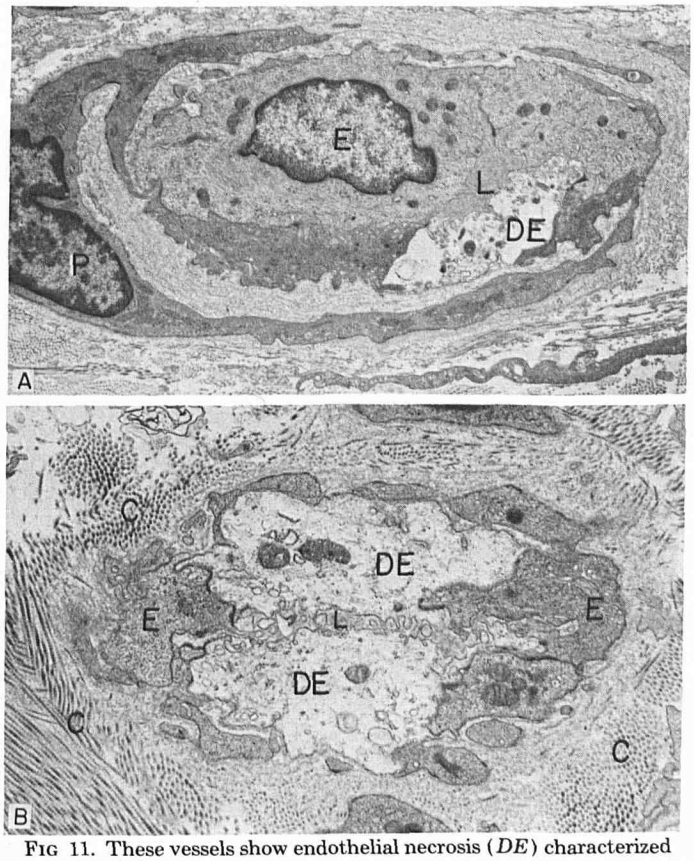

FIG 11. These vessels show endothelial necrosis (*DE*) characterized by swelling and lucency of the cytoplasm and near occlusion of the vessel lumen (*L*), as seen in *B*. In *A*, an hypertrophied endothelial cell (*E*) with numerous cytoplasmic filaments is present. Dense collagen (*C*) surrounds the vessel in *B*. *P*—pericyte. (*A*, reduced from ×8500; *B*, reduced from ×12,500).

Our ability to study on a daily basis the growth and regression of a vascularized solid tumor in the subcutaneous space of these animals cannot be duplicated in our studies of human tumors. It is also true that different mechanisms of tumor regression are operative in the same animal model. When we studied the cutaneous rejection of tumor cell suspensions in guinea pigs, the vessels showed hyperplastic microvascular changes and no evidence of endothelial injury. Rejection of tumor cells was associated with individual contact with basophils and lymphocytes. Rejection of tumor cells when the tumor suspension was given into the peritoneum was associated with lymphocytes and macrophages. These very different expressions of cellular immunity emphasize the heterogeneity of cell-mediated immunity to tumors at different sites within the same animal. It is certainly reasonable to suggest that a similar heterogeneity of response may exist in the host response to human tumors. Much like the animal model, single tumor cells may be killed by inflammatory killer cells; whereas larger areas of tumor damage may be the result of vascular necrosis and tumor infarction. Our ability to demonstrate the latter sequence, however, requires the sequential study of tumors, thereby necessitating the use of animal models, careful morphologic analysis of human tumors, and correlative conclusions based on both types of studies.

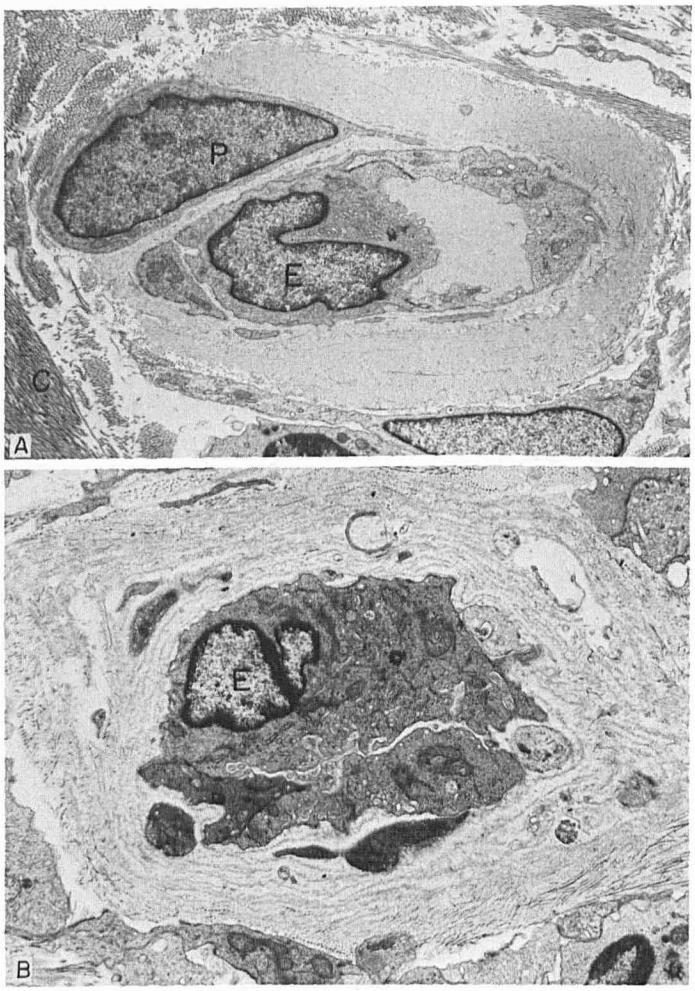

FIG 12. In *A*, this vessel shows marked thickening of the basal lamina densa. *P*—pericyte; *E*—endothelial cell. In *B*, the vascular lumen is reduced to a slit-like opening and the basal lamina shows multiple reduplications surrounding the vessel. (*A*, reduced from ×7000; *B*, reduced from ×8500).

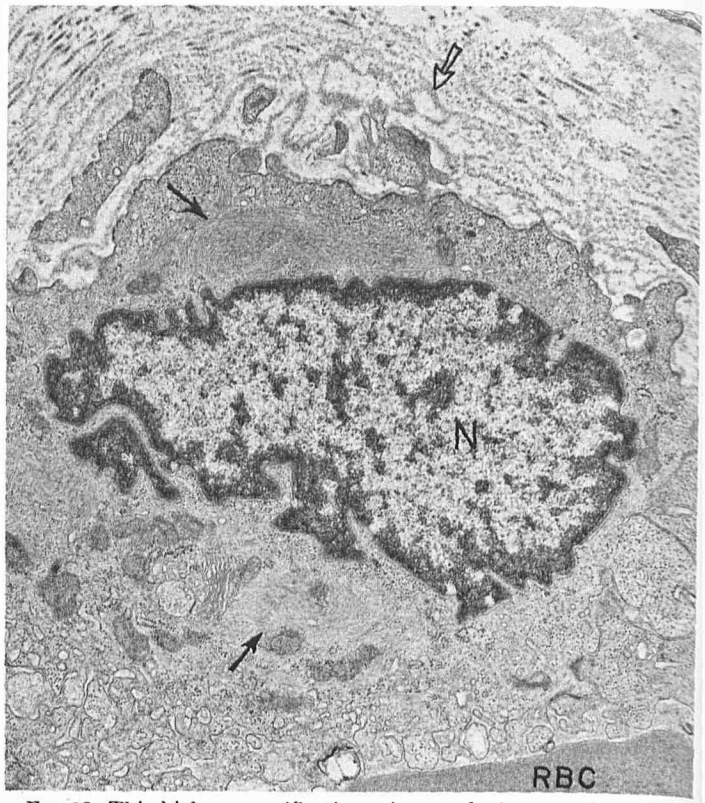

FIG 13. This higher magnification micrograph shows an hypertrophied endothelial cell with a contracted nucleus (*N*) showing nuclear pinches, masses of whorled filaments in the cytoplasm (*arrows*) and multiple irregular reduplicated fragments of underlying basal lamina (*open arrow*). A red blood cell (*RBC*) is in the vascular lumen (reduced from ×8500).

REFERENCES

1. Dvorak HF, Galli SJ, Dvorak AM: Expression of cell-mediated immunity. In vivo- Recent advances. Int Rev Experimental Pathol 21:119–194, 1980
2. Dvorak HF, Mihm MC Jr., Dvorak AM, Johnson RA, Manseau EJ, Morgan E, Colvin RB: Morphology of delayed-type hypersensitivity reactions in man. I. Quantiative description of the inflammatory response. Lab Invest 31:111–130, 1974
3. Dvorak AM, Mihm MC Jr., Dvorak HF: Degranulation of basophilic leukocytes in allergic contact dermatitis reactions in man. J. Immunol 116:687–695, 1976
4. Dvorak AM, Mihm MC Jr., Dvorak HF: Morphology of delayed-type hypersensitivity reactions in man. II. Ultrastructural alterations affecting the microvasculature and the tissue mast cells. Lab Invest 34:179–191, 1976
5. Dvorak HF, Mihm MC Jr., Dvorak AM, Barnes BA, Manseau EJ, Galli SJ: Rejection of first set skin allografts in man. The microvasculature is the critical target for the immune response. J Exp Med 150:322–337, 1979
6. Dvorak HF, Dvorak AM, Churchill WH: Immunologic rejection of diethylnitrosamine-induced hepatomas in Strain 2 guinea pigs. Participation of basophilic leukocytes and macrophage aggregates. J Exp Med 137:751–775, 1973
7. Dvorak HF, Orenstein NS, Carvalho AC, Churchill WH, Dvorak AM, Galli SJ, Feder J, Bitzer AM, Rypysc J, Giovinco P: Induction of a fibrin-gel investment: An early event in line 10 hepatocarcinoma growth mediated by tumor-secreted products. J Immunol 122:166–174, 1979
8. Dvorak HF, Dvorak AM, Manseau EJ, Wiberg L, Churchill WH: Fibrin gel investment associated with line 1 and line 10 solid tumor growth. Angiogenesis, and fibroplasia in guinea pigs. Role of cellular immunity, myofibroblasts, microvasculature damage, and infarction in line 1 tumor regression. J Natl Cancer Inst 62: 1459–1472, 1979
9. Clark WH Jr., From L, Bernardino E, Mihm MC Jr.: The histogenesis and biologic behavior of primary human malignant melanoma of the skin. Cancer Res 29:705–726, 1969

10. Spurr AR: A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43, 1969
11. Dvorak HF, Dvorak AM, Simpson BA, Richerson HB, Leskowitz S, Karnovsky MJ: Cutaneous basophil hypersensitivity II. A light and electron microscopic description. J Exp Med 132:558–582, 1970
12. Cerottini JC, Brunner KT: Cell-mediated cytotoxicity, allograft rejection and tumor immunity. Adv Immunol 18:67–132, 1974
13. Able ME, Lee JC Rosenau W: Lymphocyte-target cell interaction in vivo. Ultrastructural and cinematographic studies. Am J Pathol 60:421–434, 1970
14. Rosenau W, Moon HD: Lysis of homologous cells by sensitized lymphocytes in tissue culture. J Natl Cancer Inst 27:471–483, 1961
15. Rosenau W, Goldberg ML, Burke GC: Early biochemical alterations induced by lymphotoxin on target cells. J Immunol 111:1128–1135, 1973
16. Ruddle NH, Waksman BH: Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. J Exp Med 128:1237–1265, 1968
17. Russell SW, Rosenau W, Lee JC: Cytolysis induced by human lymphotoxin. Cinemicrographic and electron microscopic observations. Am J Pathol 69:103–118, 1972
18. Hibbs JB Jr: The macrophage as a tumoricidal effector cell: A review of in vivo and in vitro studies on the mechanism of the activated macrophage nonspecific cytotoxic reaction, The Macrophage in Neoplasia. Edited by MA Fink. Academic Press, Inc., N.Y., 1976, pp 83–111
19. Dvorak AM, Connell AB, Proppe K, Dvorak HF: Immunologic rejection of mammary adenocarcinoma (TA3-St) in C57B1/6 mice. Participation of neutrophils and activated macrophages with fibrin formation. J Immunol 120:1240–1248, 1978
20. Dvorak AM, Galli SJ, Galli AS, Hammond ME, Churchill WH, Dvorak HF: Tumor-basophil interactions in vitro- A scanning and transmission electron microscopic study. J Immunol 122: 2447–2456, 1979
21. Hibbs JB Jr.: Heterocytolysis by macrophages activated by Bacillus Calmette-Guerin: Lysosome exocytosis into tumor cells. Science 184:468–471, 1974
22. Snodgrass MJ, Hanna MG Jr.: Ultrastructural studies of histiocytic tumor cell interactions during tumor regression after intralesional injection of mycobacterium bovis. Cancer Res 33:701–716, 1973
23. Vracko R, Benditt EP: Basal lamina: The scaffold for orderly cell replacement. Observations on regeneration of injured skeletal muscle fibers and capillaries. J Cell Biol 55:406–419, 1972
24. Vracko R: Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. A review. Am J Pathol 73: 314–346. 1974

Announcement

The International Society of Tropical Dermatology will meet in Cologne April 1–4, 1981. Emphasis will be on New Developments in Dermatology with special focus on tropical and subtropical countries. Topics will include: (1) Therapy (Retinoids, Corticosteroids, Acne therapy, Leprosy); (2) Mycoses (Candidosis, Systemic mycoses, Actinomycoses); (3) Photodermatoses and Phototherapy; (4) Virology (New Therapeutic Aspects, Ticks as Transmitters, Herpes, Warts); (5) Oncology (Melanoma, Lymphoma); and (6) Contributions to actual problems and free communications.

Applications for 10 min communication must be submitted by Jan 1, 1981 to: Prof. Dr. G. K. Steigleder, Direktor der Universitäts-Hautklinik Köln, Joseph-Stelzmann-Straβe 9, D-5000 Köln 41, Germany.