THE INFLUENCE OF LOCAL HYPERTHERMIA ON LYMPHEDEMA AND LYMPHEDEMATOUS SKIN OF THE HUMAN LEG

N.F. Liu, W. Olszewski

Shanghai Institute of Plastic Surgery (NFL), Shanghai Second Medical University, Shanghai, China and Department of Surgical Research and Transplantology (WO), Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

ABSTRACT

The influence of microwave and hot water immersion hyperthermia on lymphedema and lymphedematous skin of the leg in 12 patients was studied using circumference and volumetric measurements, immunohistochemistry and "quantitative" lymphoscintigraphy. Whereas heating was associated with a reduction in the girth and volume of the leg, lymph flow as assessed by lymphoscintigraphy was unchanged. Neither absorption of the radiolabeled nanocolloid from the injection site nor the rate of tracer accumulation in the inguinal lymph nodes was significantly altered by heat therapy.

Histologically, the lymphedematous skin after heat treatment showed near resolution of perivascular cellular infiltration, disappearance of "lymph lakes" and dilatation of blood capillaries. Labeling of skin migrating immune cells with monoclonal antibodies confirmed subsidence of dermal cellular infiltration; nonetheless, there was nonspecific stimulation of resident epidermal immune cells, endothelial cells, macrophages, lymphocytes and keratinocytes by intense expression of class II and other antigens. There seemed to be a direct relationship between the subsidence of dermal inflammation and a decrease in leg edema.

We suspect that subsidence of local inflammation in the lymphedematous limb with alteration in the extracellular protein matrix after regional heating accounts for the reduction in peripheral edema.

Despite a variety of treatments for chronic lymphedema, the results are still less than satisfactory. Excision or "debulking" does not restore the physiological function of dermal lymphatics and is therefore only used in advanced conditions (1). Operations designed to reconstitute lymph flow such as lympho-venous shunting usually provide only temporary benefit and accordingly have strictly limited indications (2). Nonoperative measures such as massage, intermittent pneumatic compression, and "bandaging," on the other hand, are also therapeutically only partially effective (2).

Relatively beneficial results have been obtained using microwave hyperthermia (3-5). Most patients show a significant reduction of peripheral edema and the incidence and severity of secondary infections are sharply decreased.

This study aims to investigate the possible mechanisms of the apparent benefit of hyperthermia on lymphedematous tissue.

MATERIALS AND METHODS

Twelve patients with obstructive lymphedema of the lower extremity were studied. Four had inguinal lymph nodes removed and irradiated because of neoplastic involvement. Six had secondary lymphedema from previously repeated leg infections. The final two had obstructive lymphedema without known etiology. The longest duration of edema was 36 years and the shortest was one year.

Each patient had received some kind of earlier treatment, including antibiotics, massage, and/or a lymphovenous shunting operation. The most common treatment was external bandaging but the results were poor.

Nine of these patients underwent microwave heating of the involved leg. The microwave unit had a working frequency of 2450 MHZ. The temperature was tested using thermocouples on the skin surface, implanted subcutaneously and intramuscularly before and during the treatment. The highest temperature recorded of a treated leg was in the subcutis which reached around 40°C during treatment. The other three patients underwent hot water bath therapy. The temperature of the water was 44°C while the skin temperature during treatment was 39°C at 2mm and 37.5°C at 5mm below the surface.

The treatment time of these patients was 45 min each day for a total of 15 days as a single course, and the cycle was then repeated after a week of rest. The full treatment cycle was for 3 courses. After each treatment and

TABLE 1
Cell Phenotypes in Lymphedematous Leg Epidermis and Dermis Before and After Local Hyperthermia Compared with Healthy (Control) Leg

	Phenotype Labeling	Control	Lymphedema Before	After*
Epidermis (/lin.mm)	CD1	20-38 (31)**	40-60 (48)	20-49 (44)
	HLA-DR	23-33 (28)	8-60 (36)	24-55 (40)
Dermis (/100PVC)	CD1	+	++	+++
	CD2	0-2	18-33	2-15
	CD4	0-2	21-30	3-30
	CD8	0-2	13-30	3-20
	M718	0-2	15-16	20-25
	HLA-DR	++	++	+++
	S100	++	++	++
	M614	+	++	+++
	M407	+	++	+++
(/sq.mm)	M616	105-120 (112)	120-240 (205)	120-240 (195)

*=all in the column were more intensely stained
**=numerals in parentheses represent median values
PVC=perivascular cells; lin. mm=linear millimeter; sq. mm=square millimeter

during the intermission rest period, compression bandages were continuously worn by the patients during the day.

To evaluate heat therapy, the following features were monitored.

Skin Histology

Before and within ten days after three courses of heat treatment, a skin biopsy was taken from the anterior aspect of the calf above the ankle. One half of the specimen was stained with hematoxylin-eosin and the other half with monoclonal antibody (moab) using a two-stage immunoperoxidase technique. An array of monoclonal and polyclonal antibodies (Dakopatts, Denmark) were tested. For quantitative evaluation of cellular changes in the skin, the number of stained cells in the epidermis was counted per linear millimeter whereas in the dermis the labeled cells were counted per 100 of perivascular infiltrating cells. In the subcutaneous tissue, visual density was relied upon. All counting was blindly done by 3 pathologists independently who did not know the treatment status of each patient.

Lymphoscintigraphy

Technetium-99m labeled solco nanocoll (Switzerland) was used as the radiopharmaceutical (0.2ml or 3mci for each leg). After intradermal injection of the tracer, the patients walked at a standard speed of 3.2Km/h for 15 min to promote the transport and nodal uptake of the radiopharmaceutical. Scintiscans of lymphatics of both limbs and inguinal regions were obtained. Quantitative parameters obtained during examination were the half-life of colloid clearance (T1/2) from the injection site, lymph flow "speed" and nodal uptake rate of the tracer.

Leg limb volume was also determined by water volume displacement using a container which measured the volume of the leg at a level up to 10cm above the malleolus. The leg circumference was also measured at four points (dorsum of foot, 5cm above the internal malleolus, 15cm above and beneath the lower patella edge). The sum of the measurements was used for calculation. All parameters were measured on both legs with the contralateral nonedematous leg serving as the control. Wilcoxon's test was used for statistical analysis. $P<0.05$ refers to significance difference. The patients and data were followed up for 12 months.

RESULTS

After heating, the circumference of the treated leg decreased significantly in both groups (microwave and hot water bath) with a mean value of 11.32cm ($p<0.05$) and 8.9cm ($p<0.05$), respectively. The change in leg volume was also noteworthy with a mean reduction of 155ml ($p<0.05$) and 291ml ($p<0.05$), respectively. The greater changes of volume as compared with circumference in the water bath group was related to two patients that had more dramatic reduction of edema of the lower leg.

No patient had an attack of secondary infection during treatment and follow-up. Moreover, no "thermal burn" or other complication of heat treatment was observed.

The most common change in lymphoscintigrams were dermal backflow, few or no normal lymphatic trunks, and decreased number of inguinal nodes. After hyperthermia, there were no notable scintigraphic changes. Moreover, the quantitative studies also failed to show tracer transport differences before and after treatment. Among the 12 patients, in only 2 the clearance of tracer was faster after treatment but in 10 the clearance was unchanged or slower. A higher rate of accumulation of tracer in inguinal lymph nodes was found in 2 of 9 patients; in the remaining 7 patients, nodal tracer accumulation was less or unchanged. In one of 2 patients with greater tracer clearance from the injection site, the nodal uptake was also increased but only by 0.1%. Lymph flow speed was also not altered by heat treatment.

Before treatment, the histopathological changes in the lymphedematous skin biopsy specimens included thickening of the epidermis, "lymphatic lakes," perivascular cell infiltration, abundant fibroblasts and dense bundles of collagen. After hyperthermic therapy, the most salient histologic finding was resolution of perivascular cellular infiltration in each patient treated with microwave heating *(Fig. 1)*. Another notable change was disappearance of "lakes" of free tissue fluid *(Fig. 2)*. In some instances, dilatation of blood capillaries in the subcutis was seen.

Table 1 summarizes the skin cell phenotypes before and after hyperthermic treatment. The data of both the microwave and hot water immersion groups were amalgamated because there were only minor differences between the two patient groups. The observed phenotypic characteristics of epidermis, dermis stratum papillare and reticulare, and lymph cells were based on earlier studies performed in 30 healthy human lower leg skin specimens (control).

In the epidermis of lymphedematous skin, there were more HLA-DR antigen positive cells and they were more intensely stained after heat treatment. Of note, HLA-DR antigen on keratinocytes was expressed after but not before heat treatment *(Fig. 3)*. After heat treatment, the number of CD1 positive cells (Langerhans cells) in the epidermis was decreased but those remaining were more intensely stained *(Fig. 4)*. In the dermis, the labeling of skin migrating immune cells with moabs confirmed the subsidence of perivascular infiltration. The number of lymphocytes, which labeled with anti-CD2, CD4, CD8 antibodies was reduced. However, their staining intensity was greater after treatment. More Langerhans cells, macrophages, endothelial cells and most lymphocytes became strongly class II positive *(Fig. 3)*.

DISCUSSION

In the lymphedematous leg, hyperthermia was effective in reducing both girth and volume, decreasing recurrent attacks of inflammation, and producing histological changes including numbers and types of migrating cellular elements. A major feature of hyperthermia is to augment blood perfusion in the edematous tissue. The microwave unit used had a frequency of 2450 MHZ, with a penetration of 1.7cm, a depth suitable for an edematous extremity. During treatment, tissue temperature rose to 39-40°C, an increase which augments blood flow by a factor of 4-6 (6), maximally opens and dilates skin capillaries with subsequent increase of the surface area for filtration exchange (7). Perhaps the transient augmentation of liquid filtrate into the interstitium decreases tissue oncotic pressure which with greater microvascular exchange surface area (8) facilitates direct absorption of edema fluid. In any event, an "improved" local circulation was demonstrated by a histological reduction of "lymph lakes" and dilatation of the blood capillaries.

Increased tissue temperature alternatively may directly affect lymphatics, lymph flow and its composition. For example, in normal human leg, lymph flow increases 117% after hyperthermia at a temperature of 41°C (9). On the other hand, in chronic lymphedema an increased load of fluid and lymph in the edematous limb would have difficulty being handled by dysfunctional lymphatics with pathological changes such as fibrosis, permanent opening of intercellular junctions and atrophy of lymph nodes. In the present study, the changes in lymphatic outflow after hyperthermia were negligible. In conjunction with the unchanging lymphoscintigrams, the beneficial effects accounting for improvement of lymphedema cannot likely be accounted for by better lymph drainage.

Another possible effect of microwave treatment on lymphedematous tissue is altered tissue protein absorption by changes in macromolecular structure or composition. In lymphedema, the tissues are overloaded with plasma proteins and lipids. The thermal effect of microwave derives from oscillating free
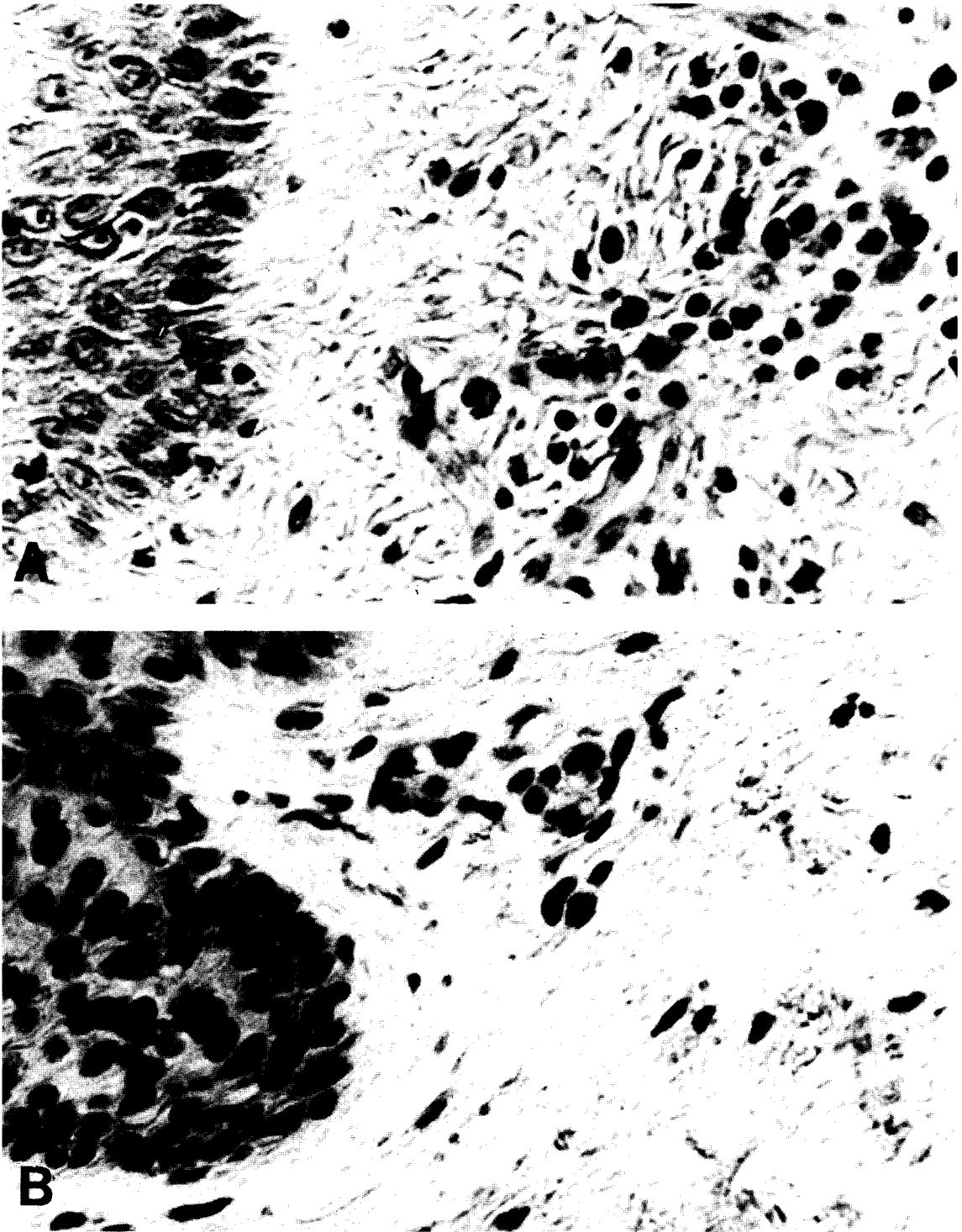
Fig. 1. Skin specimens in the postinflammatory lymphedema before (A) and after (B) microwave treatment. Note that the density of infiltrating cells in less prominent after treatment. Hematoxylin and eosin (x400).
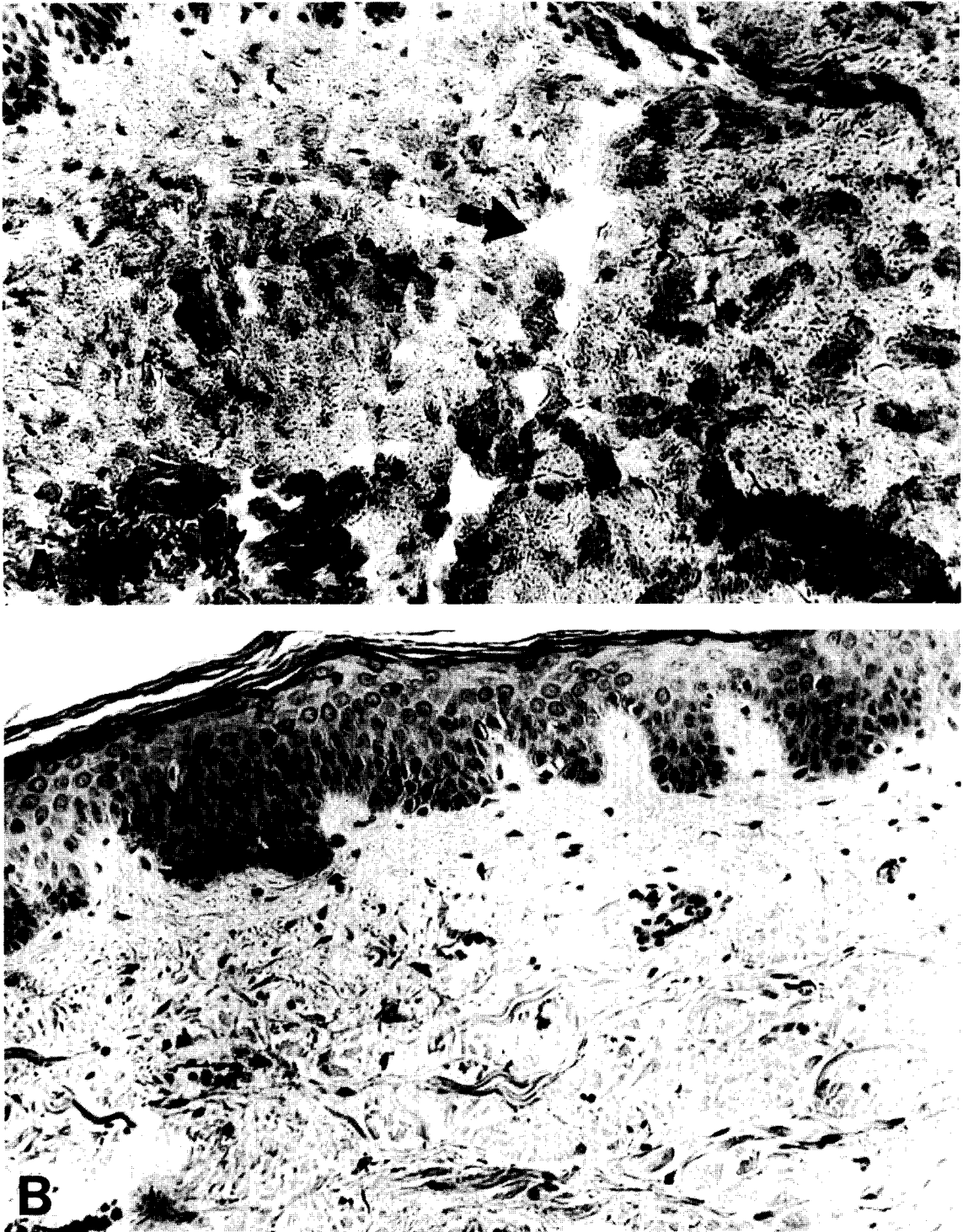
Fig. 2. Skin specimen before (A) and after (B) hyperthermic therapy. Note free lymph "lakes" in (A) (arrow), which disappeared in (B). (x100)
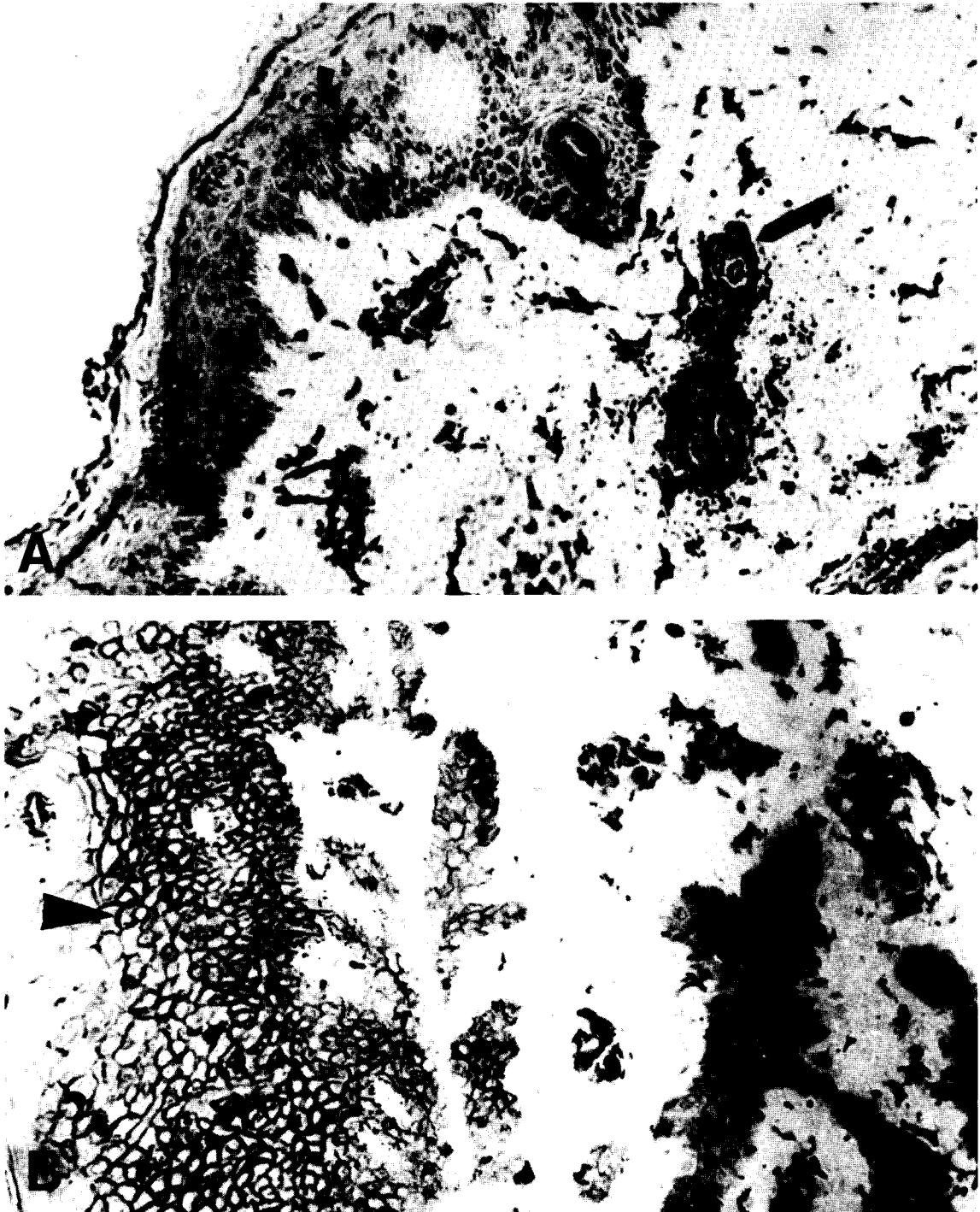
Fig. 3. Skin specimen, before (A) and after (B) microwave treatment, stained with anti-HLA-DR monoclonal antibody (moab). (A) Note positively stained Langerhans cells in epidermis and endothelial cells of blood vessels, mononuclear cells and fibroblasts in dermis (arrow) x100. (B) Intense staining of above mentioned cells and also now of keratinocytes (arrowhead) x160.
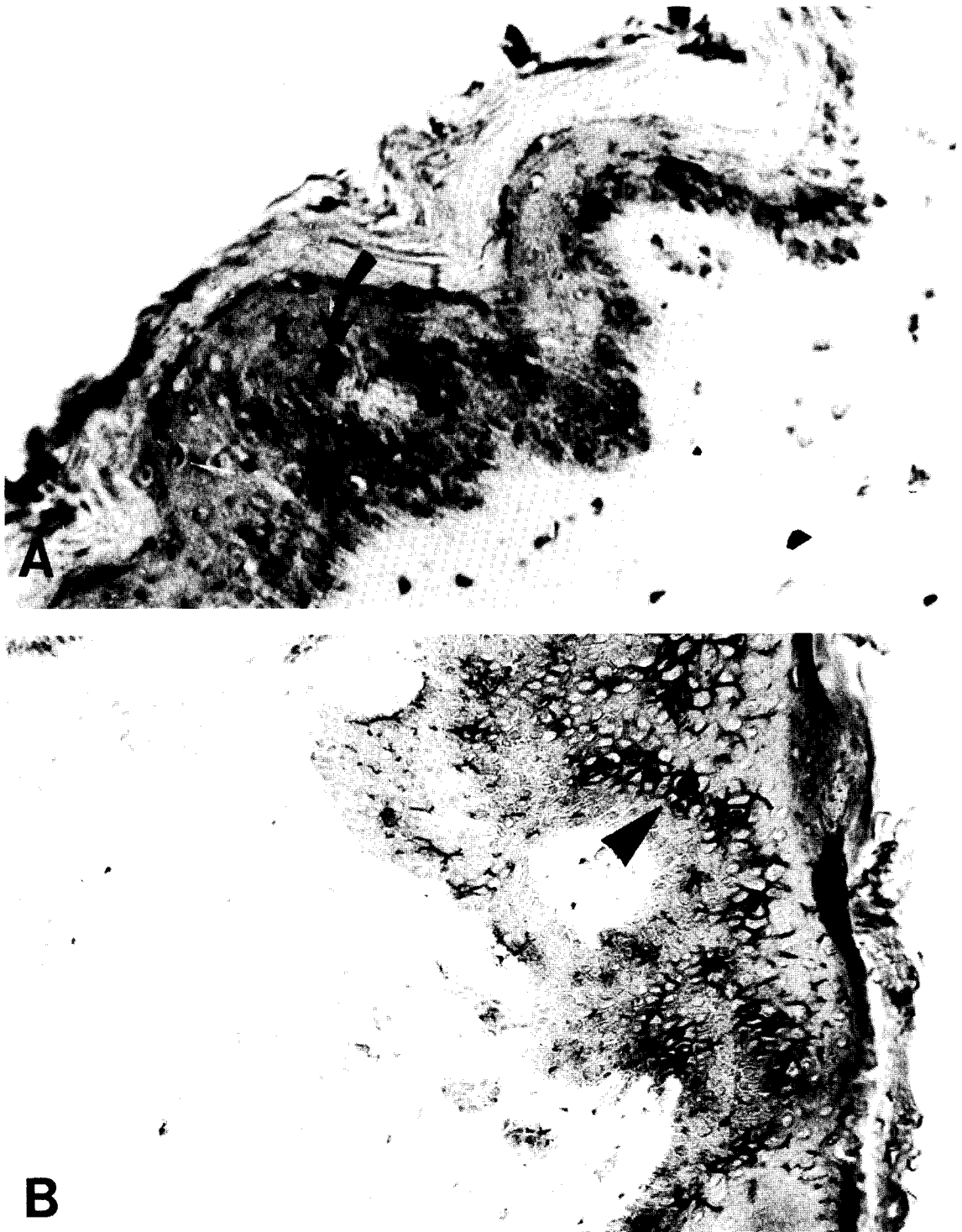
Fig. 4. Skin specimens before (A) and after (B) microwave treatment, stained with anti-CD1 moab. (A) Langerhans cells in epidermis (arrow) x250. (B) Intensively stained Langerhans cells (arrowhead) after therapy (x200).

charges or ions, rotating polar molecules and the quantum effect of excitation of molecules in the living system (10). These effects may alter large molecules and make it easier for them to be mobilized or produce internal macromolecular rearrangement with dena-turation and breakup. With heat, increased capillary infiltration of liquid may be greater than that of protein with a overall decrease in viscosity of tissue fluid. As a result, the polarized fragments of macromolecules and other wastes may more easily be transported into blood capillaries.

The main histological change in lymphedematous skin after hyperthermia was reduction of "inflammation." Routine staining with hematoxylin-eosin revealed that hyper-thermic treatment irrespective of whether induced by microwave or hot water immersion reduced mononuclear cell infiltrates around dermal blood vessels. A decrease, and in some instances disappearance of inflammatory infiltrates, paralleling subsidence of swelling suggests that local inflammation is a major component accounting for the progression of lymphedema. Immunohistochemical evalua-tion showed that hyperthermia nonspecifically stimulated resident epidermal immunocytes, endothelial cells, fibroblasts, lymphocytes and keratinocytes. Heating slightly decreased the number of epidermal Langerhans cells; on the other hand, the CD1 antigen was more strongly expressed as compared to untreated healthy control skin. Dermal Langerhans cells, macrophages, endothelial cells, and most of dermal lymphocytes became strongly class II antigen positive. Some experiments have suggested that microwave heating stimulates the immune and endocrine system (11). Local hyperthermia (12) could also stimulate the mobility of migrating cells. Parallel with the increased blood perfusion in local hyper-thermia, the egress of plasma proteins such as IgG, IgM is increased (Bryla, P, 1990, unpublished results). Heating may augment IL-1 synthesis and its secretion by keratino-cytes and Langerhans cells and perhaps also endothelial cells and fibroblasts (13). IL-1

directly activates lymphocytes and macrophages and potentiates the production of IL-2 (13). During hyperthermia IL-2 may increase interferon production and therefore enhance both macrophage and natural killer cell activity (14). The resultant increased immunological activity may effectively eliminate foreign antigens and thereby allevi-ate infectious complications in the lymphe-dematous tissue. In the long run, elimination of inflammatory factors may mitigate the influx of protein to the tissue space by decreasing capillary permeability. Thus, the most potent beneficial effect of hyperthermia may be to produce an anti-inflammatory effect. Repeated, even subliminal attacks of infection is a major cause of lymphatic damage and obstruction and subsequent progression of lymphedema. Before heat treatment, our patients had repeated attacks of leg infection, but none occurred during the treatment and follow-up time of one year. What was the fate of the lymphocytes and macrophages which had accumulated in the lymphedematous dermis and disappeared following hyperthermic therapy? Did they die or reenter the bloodstream via the venous capillaries? No evidence exists on active inward bloodstream penetration of tissue lymphocytes or macrophages back through the venous capillary wall. It also remains unclear how macromolecules can enter, if at all, the blood circulation via postcapillary venules. Whereas many of these patho-mechanisms of heat therapy remain specula-tive, the beneficial effects we observed should be further pursued.

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Liu Ningfei, M.D., Ph.D.
Shanghai Institute of Plastic Surgery
Shanghai Second Medical University
639 Zhi-Zao-Ju Road
Shanghai 200011
PEOPLE'S REPUBLIC OF CHINA