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Journal

FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 33(2)

ISSN

0892-6638

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Publication Date

2019-02-01

DOI

10.1096/fj.201801095r

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A pilot clinical trial of a near-infrared laser vaccine adjuvant: safety, tolerability, and cutaneous immune cell trafficking

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ABSTRACT: Many vaccines require adjuvants to enhance immunogenicity, but there are few safe and effective intradermal (i.d.) adjuvants. Murine studies have validated the potency of laser illumination of skin as an adjuvant for i.d. vaccination with advantages over traditional adjuvants. We report a pilot clinical trial of low-power, continuous-wave, near-infrared laser adjuvant treatment, representing the first human trial of the safety, tolerability, and cutaneous immune cell trafficking changes produced by the laser adjuvant. In this trial we demonstrated a maximum tolerable energy dose of 300 J/cm^2 to a spot on the lower back. The irradiated spot was biopsied 4 h later, as was a control spot. Paired biopsies were submitted for histomorphologic and immunohistochemical evaluation in a blinded fashion as well as quantitative PCR analysis for chemokines and cytokines. Similar to prior murine studies, highly significant reductions in CD1a$^+$ Langerhans cells in the dermis and CD11c$^+$ dermal dendritic cells were observed, corresponding to the increased migratory activity of these cells; changes in the epidermis were not significant. There was no evidence of skin damage. The laser adjuvant is a safe, well-tolerated adjuvant for i.d. vaccination in humans and results in significant cutaneous immune cell trafficking.—Gelfand, J. A., Nazarian, R. M., Kashiwagi, S., Brauns, T., Martin, B., Kimizuka, Y., Korek, S., Botvinick, E., Elkins, K., Thomas, L., Locascio, J., Parry, B., Kelly, K. M., Poznansky, M. C. A pilot clinical trial of a near–infrared laser vaccine adjuvant: safety, tolerability, and cutaneous immune cell trafficking. FASEB J. 33, 000–000 (2019). www.fasebj.org

KEY WORDS: dendritic cell · intradermal vaccination · Langerhans cell

Vaccination remains the single most important and cost-effective medical technology yet developed (1). Vaccines often require adjuvants to be effective. Adjuvants may function by anchoring antigen, by generating local inflammation, or by directly stimulating local immune cells. Few substances have proven both safe from reactogenicity and effective in amplifying immune effects; the U.S. Food and Drug Administration (FDA) has approved only 6 adjuvants over 80 yr, and the European Medicines Agency has approved only 7 adjuvants. Fewer still are ideally suited to use in the skin (2, 3), which is richly supplied with antigen-presenting cells and pain nerves (4–6). Development of new intradermal adjuvants is in keeping with the National Institute of Allergy and Infectious Diseases mission to develop new and improved vaccines against infectious diseases (7).

Recently, a novel adjuvant has been developed to be paired with intradermal vaccination, namely nondestructive laser-light stimulation of the skin (2, 8, 9). In this paper, we report the first controlled, blinded human trial of the safety and local cutaneous immune and inflammatory effects of a continuous wave (CW) near-infrared (NIR) laser adjuvant (NILA). In this pilot clinical trial of a NILA, we demonstrate the nondestructive nature of NIR laser light stimulation as well as its effects on cutaneous immune cell trafficking and local chemokine and cytokine expression.

The introduction of laser light as a vaccine adjuvant has been thoroughly reviewed elsewhere (8–12). Both laser thermotherapy (13, 14) and microporation to increase the delivery of vaccine antigen have been described (11). The use of nondestructive laser light as an intradermal vaccine adjuvant evolved from laser research in Russia, initially appearing only in an uncitable Russian publication, was

ABBREVIATIONS: APC, antigen presenting cell; CW, continuous wave; DC, dendritic cell; i.d., intradermal; hpf, high-power field; IHC, immunohistochemical; MGH, Massachusetts General Hospital; MTD, maximum tolerable dose; NILA, near-infrared laser adjuvant; NIR, near infrared; qPCR, quantitative PCR; ROS, reactive oxygen species

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doi: 10.1096/fj.201801095R

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further investigated in Western laboratories (8). Efforts in the United States have iterated this technology from expensive, bulky, poorly scalable lasers (2, 11, 14–16) to diode laser prototypes, which are much less expensive to produce and are more clinically practical. For the same reasons, CW lasers have been chosen over pulsed lasers to reduce device complexity and thus reduce cost from tens of thousands to potentially <$1000 (9, 10, 17, 18). Current lasers are used with nonpainful, nondamaging energy doses and have been carefully controlled in mouse and human studies to ensure surface temperatures below 43°C, which is the generally accepted thermal pain threshold in the skin (8, 19, 20). These cutaneous laser exposures typically cover 5–6 mm, the approximate diameter of the 0.1 ml typical intradermal (i.d.) vaccine inoculation, and become the injection sites for the i.d. immunization, which generally follows immediately. The power levels used average between 0.1 and 5 W, average power densities range from 0.5 to 5.0 W/cm^2, average exposure times are around 1 min, and radiant exposure is at most 300 J/cm^2 to limit tissue heating.

Recapitulating the original Russian approach using 532 nm visible laser illumination, in murine studies it was found that preillumination of the skin with nondestructive light below a temperature of 43°C increased cutaneous dendritic cell (DC) motility and antigen uptake of i.d. vaccine. This resulted in increased antigen presentation to draining lymph nodes, demonstrated by confocal microscopy, and was associated with significantly enhanced immune responses to 2 vaccines (2).

For the visible spectrum of light (wavelengths 380–700 nm), the major chromophores absorbing the energy of the light are melanin and hemoglobin. Above 1000 nm, water becomes the major chromophore (21), absorption by melanin and hemoglobin are reduced by 1–4 orders of magnitude, photothermal effects are thus reduced, and photochemical responses can be elicited (22). Thus, the choice of near-IR was hypothesized to be potentially independent of skin pigmentation, which is essential if the technology is to be extended to clinics world-wide. Low-power (1 mW–5 W/cm^2) NIR irradiation is now considered photobiomodulation, having temporary but profound nonpermanent physiologic effects on the underlying tissues (23, 24), including reactive oxygen species (ROS) generation.

The immune effectiveness of near-IR as a laser adjuvant was convincingly borne out in a series of recent studies. The 1064 nm CW NILA in mice resulted in increased cutaneous migratory DCs and activated $Lang^+$, $CD11b^-$ $Lang^-$ subsets of these cutaneous DCs in skin-draining lymph nodes. Activation and migration of these critical antigen-presenting cells (APCs) enabled immune amplification and dramatically increased protection from lethal viral challenge in several studies of a murine model of i.d. influenza vaccination followed by live virus respiratory influenza challenge (9, 17, 18). In additional studies (18), relative gene expression of inflammatory cytokines and chemokines in the skin were also measured. Significant changes in chemokines and immune activators were noted, particularly CCL20, CCL2, CCL11, CCL17, and CCR7, which are all known to affect DC migration and lymphocyte activation, whereas no significant change was noted in TNF-α expression in laser treated mouse skin. Kashiwagi *et al.* (9) also reported on a limited, open-label, single-arm, noninvasive safety study of the NIR laser adjuvant treatment in 5 healthy adults. That study used an FDA-approved, Q-switched Nd:YAG 1064 nm laser. In that initial limited study, skin phototypes V or VI (high melanin-containing skin types) (9, 25) were chosen. The highest irradiance was 444 J/cm^2, with no subject reporting significant discomfort and no sensation below a power density of 1.1 W/cm^2; there were no visible skin changes 2 d after irradiation (3).

The goal of this study was to demonstrate the safety, tolerability, and immune effects of a near-infrared laser device in a pilot-controlled clinical trial in which the laser-irradiated and control sites were biopsied and examined in blinded fashion immunohistopathologically for evidence of damage or immune cell changes compatible with adjuvant activity.

MATERIALS AND METHODS

The study was conducted at the Beckman Laser Institute outpatient medical clinics of the University of California Irvine (UC Irvine) and was conducted by the Department of Dermatology at UC Irvine. The study was single venue.

Study volunteers

The study enrolled healthy male and female adults ages 18–50 yr. This initial study focused on skin phototypes I and II, which are the lightest skin tones (25). Individuals with these skin tones were chosen to minimize the potential variable of melanin absorption in this initial human study. Although laser light at 1064 nm shows minimal light absorbance by melanin, it was considered possible that this wavelength might produce different thermal responses in patients with different skin phototypes, resulting in differing dosage requirements. To reduce the potential for variability in this initial pilot study, we studied skin phototypes I and II.

Excluded from the study were patients using systemic or topical steroids on the back within 30 d prior to the study. Additional exclusions were use of tanning solutions within 48 h prior to the study, history of bleeding or anticoagulation disorders, immunosuppression associated with either medication or HIV, photosensitivity, and pregnancy (excluded by hCG urine testing). Vulnerable patient populations (*e.g.*, prisoners, incompetent individuals) were also excluded.

The study protocol was approved by the UC Irvine and Massachusetts General Hospital (MGH) Investigational Review Boards (MGH IRB#2015P001434; UCI IRB #2015-1840).

Laser treatment protocol

After enrollment, a brief medical screening and review of medical history and medications was conducted. After measurement of vital signs and a physical examination, phototypes were determined and eligible patients were enrolled.

A 95% water polymer gel was applied prior to all laser treatments to all laser-exposed skin to limit excessive heat buildup within the skin. The gel was also applied to the control, nontreated skin site that was to be biopsied. Subjects and research personnel wore protective goggles appropriate to the safety rating of the laser.

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To determine the maximum tolerable laser irradiance to be used in the biopsy phase of the study, each subject received a series of 1-min exposures on 5 mm^2 areas of the skin of the lower back (**Fig. 1**) from continuous-wave 1064 nm laser light emitted by a modified handheld diode laser device (IPG Photonics, Oxford, MA, USA). Prior to the treatments, subjects were informed about the kind of skin sensations the laser may cause and were asked to indicate to the study doctor when the exposure initially became uncomfortable. In the case that an exposure produced discomfort in the subject (a tingling or pinprick sensation or uncomfortable feeling of warmth), the exposure was terminated, and that power level was recorded. Initially, 1-min treatments were given to each subject starting at 1 W/cm^2 and increasing by 1 W/cm^2 in whole number increments up to a maximum of 5 W/cm^2. If a subject experienced discomfort during any of these treatments, a second set of treatments was made between the highest tolerable and immediate preceding whole number irradiance. These treatments were done at 0.2 W/cm^2-increments (*e.g.*, 3.2, 3.4, 3.6, and 3.8 W/cm^2). The highest irradiance the subject tolerated without any discomfort for 1 min was identified as that subject's maximum tolerable dose (MTD) of irradiance. After all subjects were tested, we selected 1 W/cm^2 as the lowest irradiance that was tolerated without discomfort by all 10 subjects. This was used this in the second phase of the study.

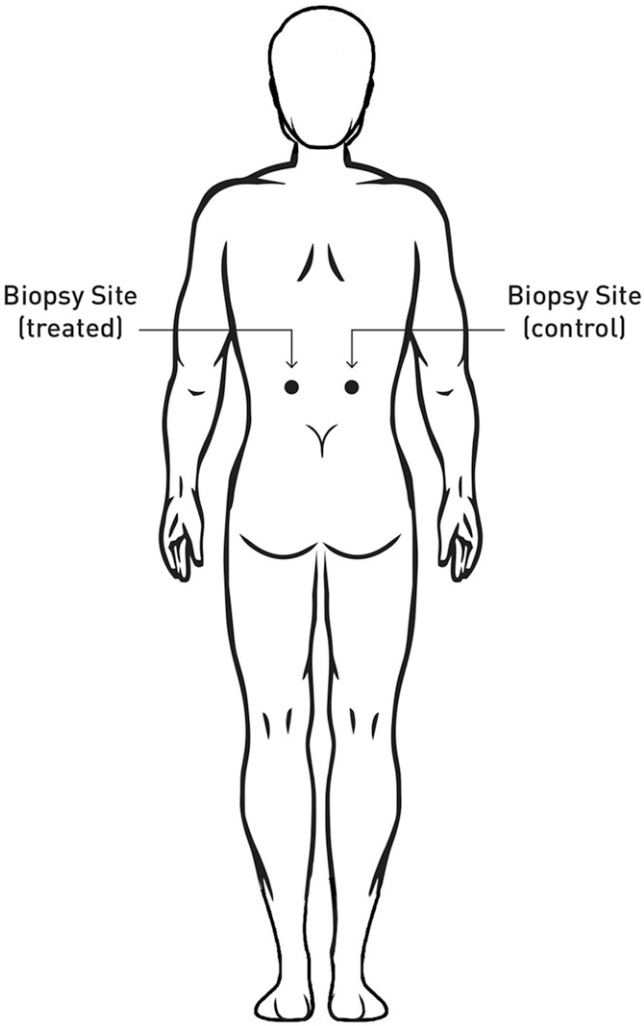

Figure 1. Location on the lower back for the biopsy sites obtained from the subjects. There was no systematic use of either the right or left sides for laser illumination. The lower back was chosen for biopsy due to its relatively lower degree of cutaneous sensitivity and cosmetic attention.

After the completion of each test, the study personnel examined the skin surface for signs of damage or alteration. Digital photographs were taken of all sites before and after laser treatment.

The determination of MTD irradiance was conducted within 12 wk of the second phase of the study. All subjects then received a single treatment to a 5-mm spot on the lower back with the CW NIR laser for 1 min at the MTD irradiance for all subjects. The laser-irradiated site and the contralateral control site were then biopsied.

Punch biopsy

Four hours after NIR irradiation, two 4-mm skin punch biopsies were performed on the skin of the lower back: one at the location of the laser exposure (experimental) and a one at a bilateral nonexposed skin site on the lower back (control). Biopsies were taken using a standard skin biopsy protocol. The skin was cleaned with alcohol, and local anesthesia (1% lidocaine with 1:100,000 epinephrine) was administered. After completion of the biopsies, routine local care was administered. The subject was seen 10–14 d after biopsy to assess the status of the biopsy site for proper healing. This clinical protocol was approved by the UC Irvine and MGH Institutional Review Boards.

Tissue analysis

Half of each biopsy sample was formalin fixed, paraffin embedded, and prepared as thin-section slides. One pair of control and treated site slides was stained with hematoxylin and eosin to evaluate microscopic changes. This analysis was conducted by faculty of the UC Irvine Department of Dermatopathology who were blinded as to whether the sample was control or laser treated. A second pair of specimen slides, identified only by subject number, was sent to MGH for further immunohistochemical (IHC) staining and analysis by a dermatopathologist (R.M.N.); control *vs.* treatment sample were blinded. The second half of each biopsy specimen was flash frozen at −80°C and couriered to MGH. These paired samples were identified only by subject number and sent to MGH for assessment of RNA expression by quantitative PCR (qPCR) for specific cytokines and chemokines. Histomorphology was assessed by light microscopy. The total number of $CD1a^+$ Langerhans cells assayed in 5 representative high-power fields (hpfs; ×400 magnification) was manually quantified within epidermis and dermis. Positive IHC staining for CD1a was defined as the presence of brown nuclear and cytoplasmic immunoreactivity quantified in areas of dense cellularity. The total number of $CD11c^+$ DCs in 5 representative hpfs was manually quantified within the dermis. Positive staining for CD11c was similarly defined. $CD1a^+$ cells are confined to the epidermis. $CD1a^+$, which is involved in antigen presentation, is the standard IHC assay for LCs (26) used by MGH Dermatopathology; CD 11^+ cells are characteristic of antigen-presenting DCs (27). Only those in the dermis were counted.

Laser-treated (case) and control skin punch biopsies were obtained and processed routinely ($n = 10$ patients). Sections (5 μm) cut from formalin-fixed, paraffin-embedded tissue blocks were stained for hematoxylin and eosin, CD1a (Langerhans cell marker; clone MTB1, Leica bond ready-to-use anti-human mouse mAb, HIER pH 9.0; Leica Biosystems, Newcastle Upon Tyne, United Kingdom), and CD11c (myeloid DC marker; clone 5D11, Novocastra bond ready-to-use anti-human mouse mAb, 1:200, HIER pH 6.0; Leica Biosystems). Appropriate controls as recommended by the manufacturer were examined.

Histomorphology was assessed by light microscopy (Olympus Corporation, Center Valley, PA, USA). The total number of

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$CD1a^+$ Langerhans cells in 5 representative hpfs (×400 magnification, 240 μm^2 round field area per field) was manually quantified within the epidermis and dermis. Positive staining for CD1a was defined as the presence of brown nuclear and cytoplasmic immunoreactivity quantified in areas of dense cellularity. The total number of $CD11c^+$ DCs in 5 representative hpfs was manually quantified within the dermis. Positive staining for CD11c was defined as the presence of brown cytoplasmic immunoreactivity in areas of dense cellularity.

Histologic sections were assessed for evidence of thermal injury [epidermal keratinocyte necrosis, hypereosinophilic keratinocyte cytoplasm, elongated keratinocyte nuclei, epidermal detachment from the basement membrane (blistering), intravascular thrombosis, hemorrhage, dermal collagen hyalinization, and dermal edema] (28, 29).

qPCR analysis of chemokine and cytokine expression in the human skin

Punch skin biopsies measuring 4 mm^2 including both the epidermis and dermis were excised 4 h after laser irradiation. Total RNA was extracted using Trizol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and the RNeasy Kit (Qiagen, Valencia, CA, USA) and reverse-transcribed using the RT^2 First Strand Kit (Qiagen). The samples were tested on an RT^2 Profiler PCR Array System (Qiagen) on a StepOnePlus PCR System (Applied Biosystems, Foster City, CA, USA). The fold change in the expression over an untreated area on the other side was normalized against housekeeping genes and calculated following the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analysis was performed using a paired, 2-tailed Student's t test. Values of $P < 0.05$ were considered statistically significant. Histologic sections were also assessed for evidence of thermal injury. For qPCR results, we ran paired Student's t tests with Bonferroni, stepdown Sidak, and false discovery rate corrections on fold increase values of laser-treated samples to the paired nontreated controls for each gene. The data analysis was conducted using SAS/STAT software for Windows (v.9.4; SAS Institute Inc., Cary, NC, USA) and Prism 7 (GraphPad Software, La Jolla, CA, USA). Correction for inflation of Type I error due to multiple significance tests was accomplished with 3 methods provided by the SAS (v.9.4) Multitest Procedure: a Sidak correction, which is conservative because it assumes tests to be corrected are independent, which is not likely to be the case here; a Bonferroni correction, which is a slightly more conservative approximation to the Sidak, which is popular because of its computational simplicity (unadjusted P values are multiplied by the number of tests performed); and false discovery rate, which provides a more liberal and powerful test at the price of allowing a certain small, acceptable estimated percentage of false-positive findings (5% in this case).

RESULTS

Clinical

The MTD of irradiance for all subjects was 1 W/cm^2; at this dose, none of the subjects had any sensation of discomfort. At a slightly higher dose (0.2 W higher), none of the subjects had any uncomfortable sensation, although 1 subject had transient erythema at the irradiation site (additional details in Supplementary Materials).

Histopathology and immunohistopathology

The paired control and laser-irradiated biopsies were evaluated by a dermatopathologist (R.M.N.) blinded as to treatment or control according to the methods previously stated. After biopsies were scored, the data for the 10 subjects was unblinded, and data from control and treatment biopsies were analyzed statistically by a paired 2-tailed Student's t test.

$CD1a^+$ Langerhans cells were less numerous within dermis as compared with epidermis in both irradiated and normal control biopsies. Compared with controls, laser treatment was associated with a reduction in the number of $CD1a^+$ Langerhans cells in both epidermis (mean ± SE of 39.0 ± 1.6 *vs.* 36.1 ± 1.6, $P = 0.24$) and dermis (21.5 ± 2.0 *vs.* 11.8 ± 1.6, $P = 0.0001$) (**Fig. 2**). Laser treatment led to a highly significant 45% reduction in Langerhans cell density within the dermis, whereas the epidermal reduction was not significant. Laser treatment was also associated with diminished Langerhans dendritic processes; this morphologic alteration of individual Langerhans cells was selectively observed within the dermal compartment (**Fig. 3**). A significant reduction in $CD11c^+$ dermal DCs (30%) was identified after laser treatment (14.8 ± 1.1 *vs.* 10.4 ± 1.4, $P = 0.0013$) (**Fig. 4**), although no definite morphologic change was seen (**Fig. 5**). No signs of thermal injury were detected.

Exploratory studies

Due to the very small size of the biopsies and the priority for immunohistochemical analysis, only 5 pairs of biopsy specimens were evaluable for qPCR analysis. Changes in gene expression in the NILA-treated biopsies showed substantial increases in CCL17 and CCL20 (**Table 1**), as were seen in mice (18). However, owing to the small sample size evaluable, none of these changes was statistically significant after Bonferroni, Sidak, and false discovery corrections.

DISCUSSION

This is the first report of a clinical trial of the safety and tolerability of the near-infrared laser adjuvant in which

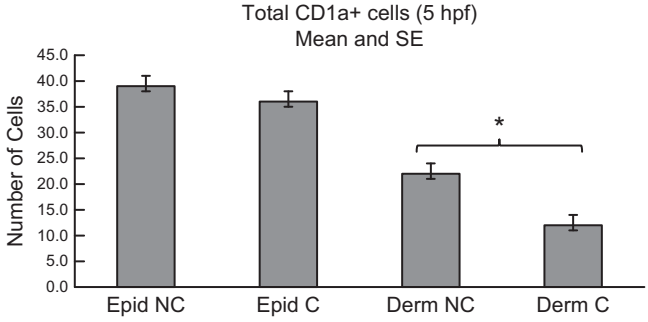

Figure 2. The total number of $CD1a^+$ Langerhans cells was reduced after laser treatment in cases (C) compared with normal controls (NC) within epidermis (Epid, P = NS) and dermis (Derm, $*P = 0.0001$) in 5 hpfs ($n = 10$ patients).

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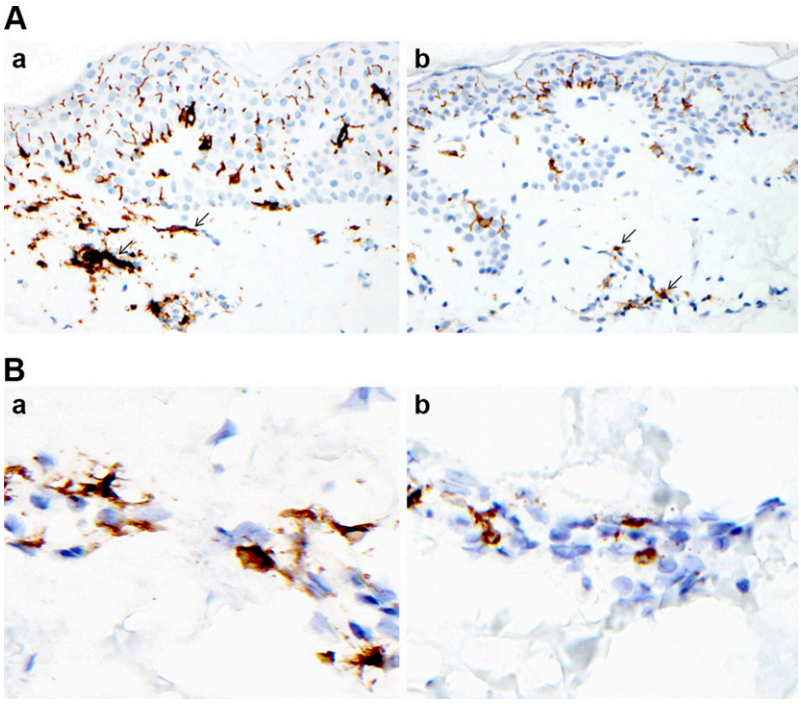

Figure 3. *A*) Subject 1840-04. CD1a stain demonstrates a significantly reduced number of Langerhans cells with diminished DC processes (arrows) selectively within the dermis in laser-treated cases (*b*) as compared with control (*a*). Original magnification, ×400. *B*) Subject 1840-09. CD1a stain demonstrated diminished DC processes in dermal Langerhans cells in laser-treated cases (*b*) as compared with control (*a*). Original magnification, ×600.

cutaneous immune functional changes were evaluated by histopathology, IHC, and gene expression of inflammatory mediators in a controlled, blinded fashion.

The laser was used as an immunologic adjuvant both in animals and in humans in the former Soviet Union and was unreported in the Western literature (8). Russian investigators noted, however, potent vaccine adjuvant effects from a copper vapor, nanosecond-pulsed laser in patients receiving i.d. influenza vaccine, hepatitis B vaccine, and cancer vaccines. Made aware of these studies on a visit to Russia, our visiting team from MGH returned to the United States to replicate and study this technology (8). We judged the copper vapor laser too expensive and impractical for broad clinical translation. Additionally, the yellow-green wavelengths absorbed by melanin did not take into account the diverse skin phototypes that would be encountered in U.S. and global populations. Studies were therefore undertaken to better understand the laser-adjuvant phenomenon and to engineer more clinically applicable devices.

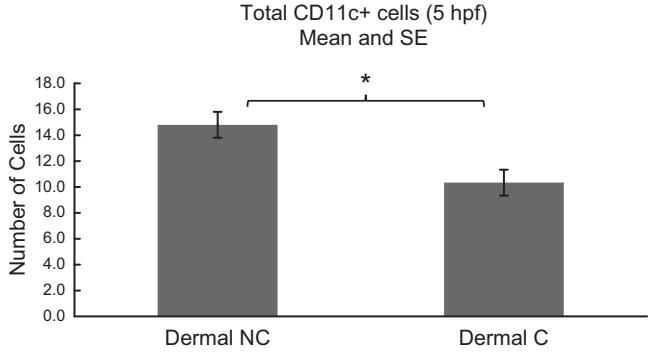

Figure 4. The total number of $CD11c^+$ dermal DCs was reduced after laser treatment in cases (C) compared with normal controls (NC) in 5 hpfs ($n = 10$ patients). *$P = 0.0013$.

Initial mouse studies used a Q-switched Nd:YAG, 532 nm nanosecond pulsed laser and demonstrated substantially enhanced antibody responses to both i.d. ovalbumin and influenza vaccines. This initial U.S. study confirmed Russian reports of increased motility of APCs, leading to increased antigen uptake as well as helper T-cell priming in draining lymph nodes (2). Humoral immune responses to influenza vaccine were increased by 400% in primary vaccination with laser when compared with the nonlaser group. Increased motility of MHC class II–positive APCs with increased migratory distance measured by intravital microscopy and increased antigen-bearing $CD11c^+$ DCs within the draining lymph nodes after i.d. vaccination with laser adjuvant as compared with vaccination without laser were also noted (2). These observations were extended with a variety of applications (9, 11, 15, 16, 30, 31).

To develop a laser adjuvant in which the parameters (wavelength, power, exposure time) would be essentially equivalent in patients of all skin pigmentation types, we examined the near-infrared spectrum of lasers. We reasoned that with essentially no melanin absorption with 1064 nm and equivalent thermal stress due to water absorption and ROS generation in all skin types (24, 32), we should still get local cell-stress inflammatory stimulation of resident APCs. Multiple animal studies of irradiances (power/unit area) delivered in minute-long exposures did not elevate temperatures over 42°C in animals and showed no evidence of histologic damage (9, 17). Studies coupling NILA with i.d. influenza vaccination in a murine model demonstrated laser-augmented influenza vaccine antibody responses (9, 17) with balanced Th1 and Th2 T-cell responses and significantly increased protection by NILA in vaccination against lethal influenza challenge. In the initial limited, open-label, single-arm, noninvasive safety study of NIR laser treatment in 5 adults of

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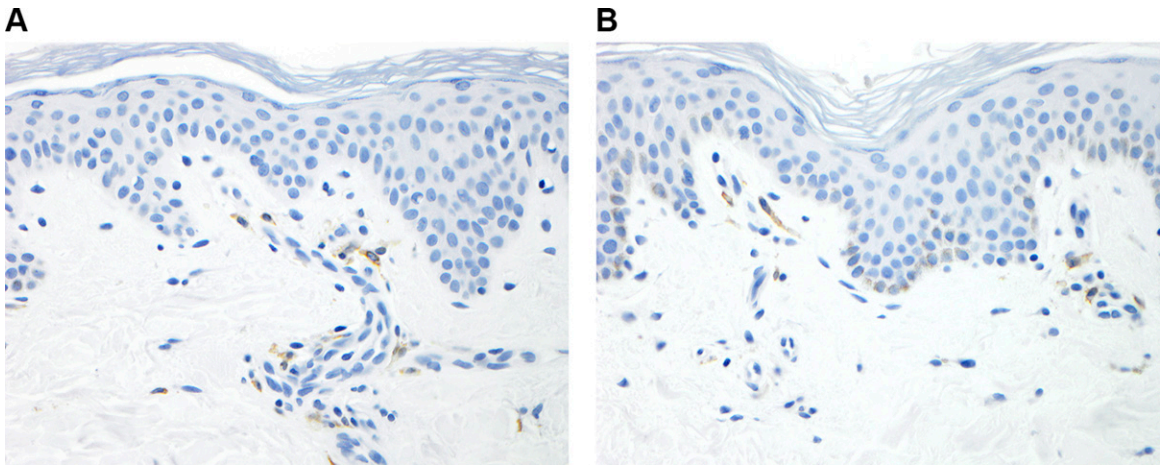

Figure 5. Subject 1840-06. Dermal dendritic cells highlighted by CD11c immunohistochemistry are reduced in number after laser treatment (*B*) as compared with control (*A*). Original magnification, ×400. The spindle-cell morphology remains unaltered.

high-melanin skin phototypes, no residual skin damage was clinically evident by 48 h (9).

Recently, Morse *et al.* (18) reported NILA increased and activated skin-resident DCs and increased DC migration into skin draining lymph nodes, quantitating the number of $FITC^+$ migratory DC populations appearing in draining lymph nodes, concluding that it was the enhanced migration responses of activated DCs that was largely responsible for enhancing immune responses to intradermal vaccination. They also demonstrated that the NILA induced selective chemokine signaling, involving CCL2, CCL17, and CCL20 in particular, without significant TNF-α induction (9). CCR7 knockout entirely inhibited migratory DC trafficking (18). NIR laser light has been documented to induce the generation of ROS in skin (33, 34), a known, albeit transient, inflammatory danger signal capable of triggering chemokine and cytokine responses, suggesting that it is the NIR laser–generated ROS that could be responsible for the local increase in DC migration that in turn results in augmented systemic immunity in the context of cutaneous vaccination (35). This also speaks to the major advantage of the NILA—namely, the absence of a persisting inflammatory stimulus as exists with current chemical adjuvants.

TABLE 1. *qPCR chemokine and cytokine changes*

Gene	Fold increase over control	SEM
CXCL13	23.5	18.1
CCL20	18.3	15.5
CCL1	12.0	4.5
CCL17	10.4	4.7
CCL11	9.7	4.2
IL-17F	5.9	4.9
IFN-G	5.9	5.2
CXCL10	5.6	2.7
IL-1B	4.9	2.9
IL-27	4.7	3.2
CCL4	4.6	3.8
IL-17C	4.3	1.9
CXCL6	4.1	2.2
CCL8	4.0	3.6
TNF-SF11	4.0	2.7
CSF3	3.6	3.3
CCR4	3.3	2.3
IL-8	3.1	2.4
VEGFA	3.1	2.2

The changes in expression of cytokines and chemokines as measured by fold increases in laser-treated skin over baseline, control skin as measured in biopsied skin. Changes >3-fold over control are shown. None of the changes is statistically significant due to the very small sample size because of the limited tissue available.

There are some substantial differences between the mouse and human findings that are likely explained by host size, distance, and time. In the murine studies, biopsies were obtained 6 h after vaccination, and the illuminated site had increased numbers of APCs. In the human studies, biopsies were obtained 4 h after laser treatment for the convenience and comfort of the subjects. In both the murine and human studies, however, the outcome was increased DC migration toward the draining lymph nodes (2, 18), a process that is accelerated by antigen and yet more by antigen augmented by the laser adjuvant (2). Finally, the human biopsy data on gene expression, though not statistically significant, which may be the result of the limited material available, is consistent with the conclusion from murine studies that there is laser-stimulated cutaneous signaling primarily involving chemokines and with minimal inflammatory cytokine expression.

The largest increase in chemokine expression was in CXCL13 (23.5 ± 18.1-fold). This chemokine and its receptor play critical roles in the maturation and generation of high-affinity–matured antibody responses in the germinal centers of lymph nodes (36). This is a desirable target effect for a vaccine adjuvant to have on draining nodes because CCL17 and CCL20 are both involved in cross-priming cytotoxic T lymphocytes (37, 38), another potentially useful effect for an adjuvant.

This is the first report of a pilot clinical trial of the safety and tolerability of the continuous-wave NILA for vaccines, in which cutaneous immune functional changes were evaluated by histopathology, IHC, and gene expression of inflammatory mediators in a controlled, single-blinded

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fashion. In this pilot study of a 1064 nm CW NIR laser, we have documented the laser adjuvant effect on cutaneous immune cell trafficking. Using a blinded observer for dermatopathology evaluation, we have demonstrated statistically significant increases in APC and cutaneous DC migration produced by nonpainful, nondestructive laser illumination. These changes included highly significant decreases in dermal Langerhans cells and dermal DCs in the targeted skin 4 h after laser illumination, corresponding to the increased migration of dermal DCs to draining lymph nodes with the up-regulation of a selective set of immune-potentiating cytokines, as repeatedly demonstrated in murine studies. As with the mouse studies, there was no corresponding increase in TNF-α generation in the NIR-irradiated human skin and no histochemical or clinical evidence of cutaneous damage.

There are significant advantages of using i.d. vaccination over the i.m. route of administration (39, 40). For example, i.d. vaccination involves less pain from vaccination because i.d. vaccination involves either smaller needles or microneedle patches, advancing toward the clinic (41), and i.d. vaccination promises to enhance vaccine responses, providing a more direct route to the immune system through cutaneous DCs and draining cutaneous lymphatics. Furthermore, i.d. vaccination is typically antigen sparing (39, 40). Conventional adjuvants are typically inappropriate for i.d. vaccination. The near-infrared laser adjuvant generates a temporary, ephemeral ROS danger signal, leaving no residual inflammatory chemical compound in the skin. This produces neither pain nor evidence of permanent cellular damage, rather exerting its immune stimulating effect through activation of chemokines and activation and migration of APCs. What has been missing to date has been acceptable skin-compatible vaccine adjuvant technologies capable of being modulated. The exquisitely controllable dosimetry of laser illumination enables meticulous modulation and merits further investigation of its adjuvant capabilities. FJ

ACKNOWLEDGMENTS

The authors thank Patricia Della Pelle (MGH Histopathology Core) for excellent technical support. This work was supported by the U.S. National Institutes of Health, National Institute of Allergy and Infectious Diseases (Grants R41AI114012, R42AI114012, and R01AI105131 to S.K.); the MGH Vaccine and Immunotherapy Center Innovation Fund; the Edmund C. Lynch, Jr. Cancer Fund; and the Seth Sprague Foundation. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

J. Gelfand, R. M. Nazarian, T. Brauns, E. Botvinick, B. Parry, K. M. Kelly, and M. C. Poznansky designed the study; R. M. Nazarian, S. Kashiwagi, B. Martin, Y. Kimizuka, K. Elkins, L. Thomas, and K. M. Kelly performed the studies; R. M. Nazarian, S. Kashiwagi, Y. Kimizuka, S. Korek, and J. Locascio analyzed the data; and J. Gelfand, T. Brauns, and M. C. Poznansky wrote the manuscript.

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Received for publication June 8, 2018.
Accepted for publication August 6, 2018.

sebj.org by Uhiv of California-Irvine Science Library Serials (128.200.102.34) on October 25, 2018. The FASEB Journal Vol. ${article.issue.getVolume()}, No. ${article.issue.getIssueN