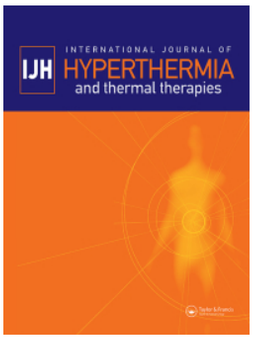

International Journal of Hyperthermia

ISSN: 0265-6736 (Print) 1464-5157 (Online) Journal homepage: informahealthcare.com/journals/ihyt20

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To cite this article: Qing Chen & Sharon S. Evans (2005) Thermal regulation of lymphocyte trafficking: Hot spots of the immune response, International Journal of Hyperthermia, 21:8, 723-729, DOI: 10.1080/02656730500271734

To link to this article: https://doi.org/10.1080/02656730500271734

Published online: 13 May 2011.

Int. J. Hyperthermia, December 2005; 21(8): 723–729

Thermal regulation of lymphocyte trafficking: Hot spots of the immune response

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(Received 6 May 2005; accepted 21 June 2005)

Abstract
Lymphocytes use extensive vascular networks to traffic to various destinations in the body, including lymphoid organs and extra-lymphoid tissues. This discussion will focus on the emerging evidence that thermal stress regulates the traffic signals that direct the exit of lymphocytes from the vascular freeway. This issue is particularly relevant to T cell-based cancer immunotherapy where delivery of immune effector lymphocytes to neoplastic lesions depends on their extravasation across tumour micro-vessels. Although tumours are frequently highly vascularized by vessels that are competent to support blood flow, the tumour micro-environment has been characterized as non-permissive to lymphocyte extravasation. This may lead to a scenario where limited leukocyte infiltration at tumour sites correlates with a poor prognosis. These observations support the thesis that adjuvant strategies that promote trafficking of tumour-reactive cytolytic leukocytes to tumour sites have the potential to improve the efficacy of immune-based cancer therapy.

Keywords: *Lymphocyte, trafficking, thermal stress, fever, tumour microvessels*

Relationship between endothelial adhesion and lymphocyte trafficking

In considering the mechanisms that potentially limit lymphocyte trafficking to tumour sites, it is instructive to compare and contrast adhesion events at neoplastic sites with the molecularly well-defined mechanisms that control the egress of blood-borne lymphocytes into peripheral lymphoid organs. In lymph nodes and Peyer's patches, lymphocyte extravasation occurs preferentially across specialized vessels termed high endothelial venules (HEV) [1–4]. The efficiency of this physiological process is revealed by estimates that $\sim 5 \times 10^6$ lymphocytes continuously extravasate through HEV per second in humans [1]. HEV are lined by cuboidal endothelial cells that are demarked by high level expression of the CD31 pan-endothelial adhesion molecule (Figure 1(a)). HEV are morphologically and biochemically distinguished from squamous endothelium found in the majority of vessels

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ISSN 0265–6736 print/ISSN 1464–5157 online
DOI: 10.1080/02656730500271734

in extra-lymphoid organs where limited lymphocyte extravasation occurs under basal conditions [1–3]. Notably, local injury or inflammation induces squamous endothelium to undergo transformation into HEV-like vessels that are a locus for leukocyte recruitment at extra-lymphoid sites [1].

Lymphocyte extravasation across HEV in lymphoid organs involves highly ordered, step-wise adhesive interactions [1–4]. In this context, endothelial adhesion molecules and chemokines function as traffic signals that control cellular transit through vascular roadways. Overall, it is estimated that 25% of lymphocytes that enter HEV successfully complete the full sequence of adhesion events culminating in extravasation [4]. In peripheral lymph nodes, free-flowing lymphocytes (travelling with a 'green light') enter lymph node HEV and convert to slow-rolling cells ('yellow light') through L-selectin-dependent tethering and rolling interactions with sialomucin-like adhesion molecules on vessel walls. In Peyer's patches, a second lymphocyte homing receptor, $\alpha 4\beta 7$ integrin, also contributes to tethering and rolling events in HEV. In both lymph nodes and Peyer's patches, HEV presentation of the CC chemokine ligand (CCL)21 (TCA-4/SLC/6C-kine/exodus-2) to the CCR7 chemokine receptor on apposing naïve and central memory lymphocytes stimulates the transition from rolling cells to firmly adherent cells ('red light'). Firm arrest and subsequent extravasation across the endothelial barrier are mediated by binding of leukocyte function adhesion molecule-1 (LFA-1) to its endothelial receptors inter-cellular adhesion molecule (ICAM)-1 (Figure 1(a)) and ICAM-2.

Tumour vessels of murine tumours or human patients are generally flat-walled, in contrast to the typical cuboidal morphology of HEV in lymphoid organs (Figure 1(a, b)) [5–7]. Consistent with these findings, *in vivo* imaging techniques using intra-vital microscopy indicate that lymphocytes interact poorly with tumour vessels under haemodynamic shear [6, 8, 9]. One explanation for limited lymphocyte interactions in tumour vessels is the comparatively low level of expression of endothelial adhesion molecules (e.g. ICAM-1) and chemokines that are hallmarks of extravasation or inflammation (Figure 1(a, b)) [5, 6]. Notably, the failure of lymphocytes to infiltrate tumour tissues has been correlated with an unfavourable prognosis in melanoma, lung and ovarian cancer patients and in numerous mouse models (Figure 1(b)) [5, 6, 10, 11]. These observations support the notion that novel strategies are needed to improve lymphocyte trafficking to neoplastic tissues. This issue is particularly important in the development of effective vaccine therapies where immunization aims to increase the frequency of tumour-reactive cytotoxic T cells (CTL). A frequently overlooked consideration is that optimal vaccine therapy is equally dependent on tumour-specific CTL gaining access to tumour tissues in order to be effective in killing targets by contact-mediated lysis.

Stimulation of lymphocyte-endothelial cell interactions by thermal stress

Multiple lines of evidence indicate that thermal stress in the form of therapeutic hyperthermia can control lymphocyte-endothelial adhesion. The role of high temperature, short duration heat shock in regulating adhesion has principally been investigated by *in vitro* models for resting, non-activated squamous endothelial cells, i.e. human primary endothelial cell cultures including macro-vascular HUVEC (human umbilical vein endothelial cells) and micro-vascular HMVEC (human micro-vascular endothelial cells). Direct exposure of HUVEC or HMVEC to heat shock (e.g. 43°C for 2 h) increases endothelial cell expression of ICAM-1 molecules [12, 13]. Interestingly, several studies have reported that the major cellular products of heat shock, i.e. heat shock proteins (HSP), can act extra-cellularly to stimulate endothelial adhesion. In this regard, recombinant

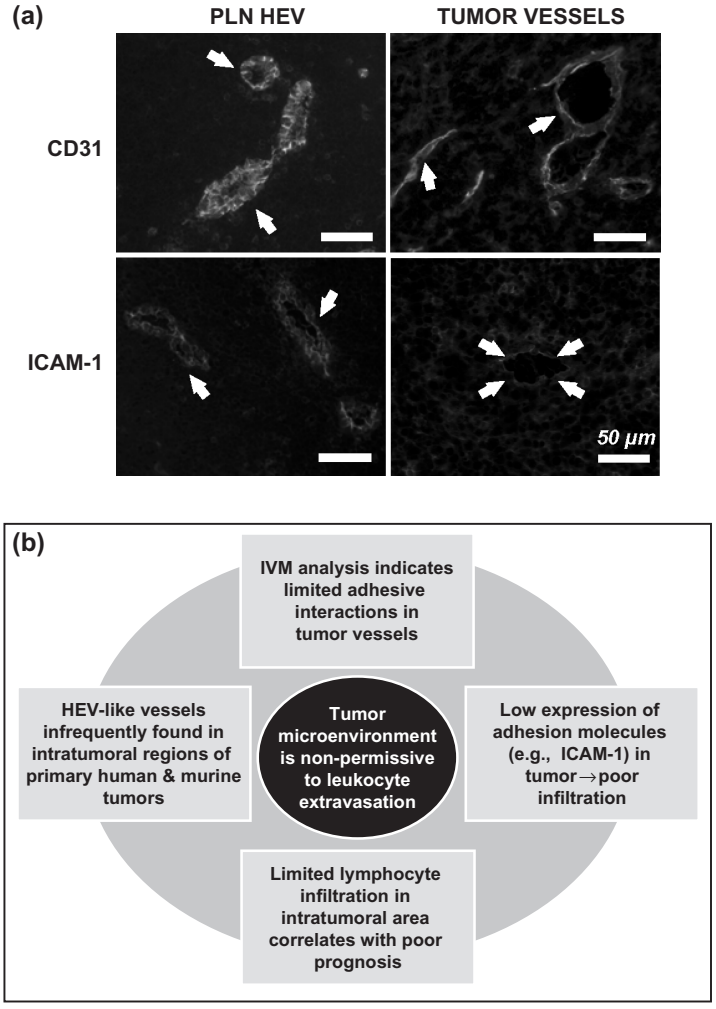

Figure 1. Comparative analysis of lymphoid organ HEV and tumour vessels. (a) Cryosections of peripheral lymph nodes (PLN) and transplanted colon 26 tumours from the same experimental mouse were stained with CD31-specific monoclonal antibody (mAb; 5 μg ml^{-1}) and FITC-labelled secondary antibody. ICAM-1 expression on the luminal surface of vessels was detected by intravenous injection of ICAM-1-specific mAb (500 μg per mouse) into colon 26 tumour-bearing mice. Tissues were harvested after 20 min and cryosetions were stained with TRITC-labelled secondary antibody. Immunofluorescent images were captured using the same exposure time in order to compare the relative expression levels of adhesion molecules by vessels. Arrows indicate cuboidal HEV in PLN or flat-walled squamous vessels in tumour tissues. ICAM-1 staining is low on tumour vessels compared to PLN HEV. Note that staining of ICAM-1 on tumour cells is also evident, which may result from access of ICAM-1 mAb to tissues outside the vascular compartment due to the inherent leakiness of tumour vessels [7]. (b) Schematic shows proposed inter-relationship between the parameters that limit lymphocyte-endothelial adhesion in the tumour micro-environment and clinical outcome.

HSP (HSP60, HSP65 or HSP70) of mammalian or bacterial origin act on HUVEC through an NF-κB signalling pathway to induce ICAM-1 expression and augment the ability to support lymphocyte adhesion [14–16]. One of the interesting findings to emerge is that increased shear forces also stimulate ICAM-1 expression by HUVEC *in vitro* [17].

This may be relevant during hyperthermia responses in tissues when haemodynamic shear is influenced by changes in blood flow [18]. While the effects of heat shock or haemodynamic shear on endothelial adhesion have not been explored *in vivo*, it is tempting to speculate, based on *in vitro* endothelial systems, that high temperature thermal therapy could elicit changes in vascular adhesion and lymphocyte homing if delivered locally to tumour sites.

A recent series of *in vivo* studies have established that fever-range thermal therapy enhances lymphocyte-endothelial adhesion and site-specific trafficking [6, 19, 20]. An intriguing aspect is that fever-range thermal stress promotes homing by inducing independent changes in adhesion in both lymphocytes and target endothelium. In this regard, exposure of lymphocyte sub-sets (including naïve and central memory lymphocytes and $CD4^+$ and $CD8^+$ T cells) to febrile-range temperatures (38–40°C for 6 h) *in vitro* or *in vivo* stimulates the binding activity of lymphocyte homing receptors including L-selectin and $\alpha 4\beta 7$ integrin [19, 21–25]. This results in enhanced trafficking of lymphocytes across HEV in lymphoid organs [21, 26]. Thermal stimulation of lymphocyte adhesion was found to be dependent on the pro-inflammatory cytokine, interleukin-6 (IL-6), through its ability to initiate MEK-1/ERK-1-2 signalling downstream of the gp130 signal transduction molecule [25].

The adhesive properties of endothelial cells in HEV of lymphoid organs have further been shown to be amplified by fever-range whole body thermal therapy [26, 27]. Thus, elevation of mouse core temperatures to the febrile range (39.5–40°C for 6 h), using procedures originally developed by Dr. Repasky's laboratory [27], augments the capacity of HEV to support lymphocyte adhesion under shear and promotes lymphocyte trafficking to lymph nodes and Peyer's patches [19, 20, 26]. Enhanced lymphocyte trafficking to secondary lymphoid organs increases the probability that lymphocytes encounter cognate antigens within a micro-environment conducive to generating optimal adaptive immune responses. Related studies indicate that lymphocyte-endothelial adhesion is similarly enhanced in tumour vessels by fever-range thermal therapy (Q. Chen and S. S. Evans, unpublished observations). Notably, thermal stress does not promote changes in adhesion or trafficking across squamous, resting endothelium of non-malignant tissues *in vivo* [6, 26]. These observations suggest that control of vascular adhesion is influenced by exquisitely regulated molecular events within unique tissue micro-environments of lymphoid organs, tumour tissues and extra-lymphoid organs. Site-specific control of endothelial adhesion serves to focus the delivery of immune effectors cells to physiologically relevant tissues while preventing inappropriate encounters between cytolytic lymphocytes and normal tissues.

Conclusions and future directions

The emerging data support the concept that immune surveillance is promoted by physiological febrile responses that accompany infection and inflammation by virtue of the ability to improve lymphocyte trafficking across lymphoid organ HEV (Figure 2). Optimal immune protection is maintained under non-pathologic conditions since fever-range thermal stress does not indiscriminately amplify lymphocyte migration across squamous endothelium of all tertiary organs. Further study is required to determine if local or whole body thermal therapy can invoke similar adhesive mechanisms within heterogeneous tumour micro-environments, thereby augmenting delivery of tumour-specific $CD8^+$ cytotoxic T cells (Figure 2). Enhanced infiltration by $CD8^+$ T cells in primary and secondary lesions would be predicted to lead to improved tumour growth

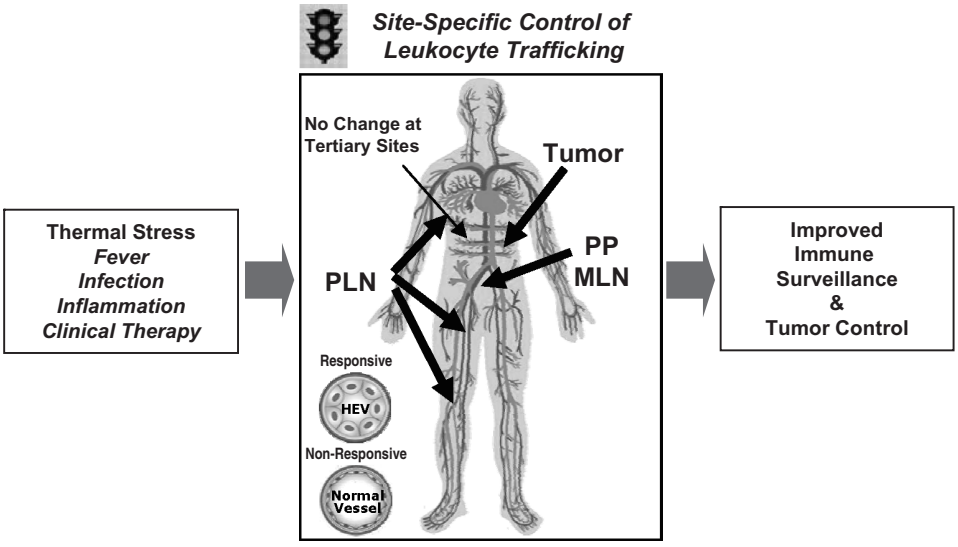

Figure 2. Model for thermal regulation of traffic signals that direct site-specific extravasation of lymphocytes across vascular beds. Thermal stress induced by inflammation or clinical therapy promotes lymphocyte trafficking across cuboidal HEV in lymphoid organs (PLN, mesenteric LN [MLN] and Peyer's Patches [PP]) and potentially in tumour vessels. In contrast, lymphocyte-endothelial adhesion and trafficking across non-activated squamous endothelium of extra-lymphoid organs is not augmented by fever-range thermal stress under non-pathological conditions. Thus, site-specific control of vascular adhesion by thermal stress has the potential to enhance immune surveillance and limit tumour progression.

control in immunologically responsive malignancies. The underlying mechanisms by which thermal stress stimulates endothelial adhesion within a select sub-set of blood vessels remain to be addressed. Likely candidate effector mechanisms are pro-inflammatory cytokines, since these molecules are known to be potent stimulators of endothelial adhesion. Additional investigation is required to determine the mode of thermal regulation of vascular endothelial adhesion. Resolution of this question depends on the identification of the specific traffic signals (i.e. adhesion molecules and chemokines) that are targeted by thermal stress in addition to mapping of the signal transduction pathways responsible for improving the delivery of lymphocytes across vascular roadways.

Acknowledgements

We thank Elizabeth Repasky and Heinz Baumann for insightful discussions relating to this work and current and previous laboratory members for numerous contributions to this investigation. This work was supported by grants from the NIH (CA79765, CA094045, CA16056), the Department of Defense (W81XWH-04-1-0354), the Roswell Park Alliance Foundation and the Buffalo Foundation.

References

1. Girard JP, Springer TA. High endothelial venules (HEVs): Specialized endothelium for lymphocyte migration. Immunology Today 1995;16:449–457.

2. Butcher EC, Williams M, Youngman K, Rott L, Briskin M. Lymphocyte trafficking and regional immunity. Advances in Immunology 1999;72:209–253.
3. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. Natural Reviews in Immunology 2003;3:867–878.
4. Miyasaka M, Tanaka T. Lymphocyte trafficking across high endothelial venules: Dogmas and enigmas. Natural Reviews in Immunology 2004;4:360–370.
5. Chen Q, Wang WC, Evans SS. Tumor microvasculature as a barrier to antitumor immunity. Cancer Immunology & Immunotherapy 2003;52:670–679.
6. Chen Q, Fisher DT, Kucinska SA, Evans SS. Dynamic control of lymphocyte trafficking by fever-range thermal stress. Cancer Immunology & Immunotherapy (in press).
7. Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM. Openings between defective endothelial cells explain tumor vessel leakiness. American Journal of Pathology 2000;156:1363–1380.
8. Jain RK, Munn LL, Fukumura D. Dissecting tumour pathophysiology using intravital microscopy. Natural Reviews in Cancer 2002;2:266–276.
9. Wu NZ, Klitzman B, Dodge R, Dewhirst MW. Diminished leukocyte-endothelium interaction in tumor microvessels. Cancer Research 1992;52:4265–4268.
10. Carlos TM. Leukocyte recruitment at sites of tumor: Dissonant orchestration. Journal of Leukocyte Biology 2001;70:171–184.
11. Ganss R, Arnold B, Hammerling GJ. Mini-review: Overcoming tumor-intrinsic resistance to immune effector function. European Journal of Immunology 2004;34:2635–2641.
12. Lefor AT, Foster CE 3rd, Sartor W, Engbrecht B, Fabian DF, Silverman D. Hyperthermia increases intercellular adhesion molecule-1 expression and lymphocyte adhesion to endothelial cells. Surgery 1994;116:214–220; discussion 220–221.
13. Shah A, Unger E, Bain MD, Bruce R, Bodkin J, Ginnetti J, Wang WC, Seon B, Stewart CC, Evans SS. Cytokine and adhesion molecule expression in primary human endothelial cells stimulated with fever-range hyperthermia. International Journal of Hyperthermia 2002;18:534–551.
14. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. Journal of Clinical Investigations 1999;103:571–577.
15. Galdiero M, de l'Ero GC, Marcatili A. Cytokine and adhesion molecule expression in human monocytes and endothelial cells stimulated with bacterial heat shock proteins. Infection & Immunology 1997;65:699–707.
16. Verdegaal ME, Zegveld ST, van Furth R. Heat shock protein 65 induces CD62e, CD106, and CD54 on cultured human endothelial cells and increases their adhesiveness for monocytes and granulocytes. Journal of Immunology 1996;157:369–376.
17. Chien S, Li S, Shyy YJ. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. Hypertension 1998;31:162–169.
18. Vujaskovic Z, Song CW. Physiological mechanisms underlying heat-induced radiosensitization. International Journal of Hyperthermia 2004;20:163–174.
19. Appenheimer MM, Chen Q, Girard RA, Wang WC, Evans SS. Impact of fever-range thermal stress on lymphocyte-endothelial adhesion and lymphocyte trafficking. Immunological Investigations 2005;34:295–323.
20. Chen Q, Clancy KA, Wang WC, Evans SS. High endothelial venules: Master regulators of lymphocyte trafficking and targets of fever-range thermal stress. In: Aird W, editor. The endothelium: A comprehensive reference. Cambridge University Press (in press).
21. Wang WC, Goldman LM, Schleider DM, Appenheimer MM, Subjeck JR, Repasky EA, Evans SS. Fever-range hyperthermia enhances L-selectin-dependent adhesion of lymphocytes to vascular endothelium. Journal of Immunology 1998;160:961–969.
22. Evans SS, Frey M, Schleider DM, Bruce R, Wang WC, Repasky E, Appenheimer MM. A Regulation of leukocyte-endothelial cell interactions in tumor immunity. In: Mihich H, Croce C, editors. The biology of tumors. New York: Plenum Press; 1998. pp 273–286.
23. Evans SS, Schleider DM, Bowman LA, Francis ML, Kansas GS, Black JD. Dynamic association of L-selectin with the lymphocyte cytoskeletal matrix. Journal of Immunology 1999; 162:3615–3624.
24. Evans SS, Bain MD, Wang WC. Fever-range hyperthermia stimulates $\alpha 4\beta 7$ integrin-dependent lymphocyte-endothelial adhesion. International Journal of Hyperthermia 2000;16:45–59.

25. Chen Q, Wang WC, Bruce R, Li H, Schleider DM, Mulbury MJ, Bain BD, Wallace PK, Baumann H, Evans SS. Central role of IL-6 receptor signal-transducing chain gp130 in activation of L-selectin adhesion by fever-range thermal stress. Immunity 2004;20:59–70.
26. Evans SS, Wang WC, Bain MD, Burd R, Ostberg JR, Repasky EA. Fever-range hyperthermia dynamically regulates lymphocyte delivery to high endothelial venules. Blood 2001;97:2727–2733.
27. Pritchard MT, Ostberg JR, Evans SS, Burd R, Kraybill W, Bull JM, Repasky EA. Protocols for simulating the thermal component of fever: Preclinical and clinical experience. Methods 2004;32:54–62.