Published in final edited form as:

Wound Repair Regen. 2008 ; 16(3): 442–449. doi:10.1111/j.1524-475X.2007.00351.x.

Tissue profiling MALDI mass spectrometry reveals prominent calcium-binding proteins in the proteome of regenerative MRL mouse wounds

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Abstract

MRL/MpJ-Fas^{lpr} mice exhibit the ability to regenerate ear tissue excised by dermal punches. This is an exceptional model to identify candidate proteins that may regulate regeneration in typically nonregenerative tissues. Identification of key molecules involved in regeneration can broaden our understanding of the wound-healing process and generate novel therapeutic approaches. Tissue profiling by matrix-assisted laser desorption ionization mass spectrometry is a rapid, powerful proteomic tool that allows hundreds of proteins to be detected from specific regions of intact tissue specimens. To identify these candidate molecules, protein expression in ear punches was examined after 4 and 7 days using tissue profiling of MRL/MpJ-Fas^{lpr} mice and the nonregenerative mouse strain C57BL/6J. Spectral analysis revealed distinct proteomic differences between the regenerative and nonregenerative phenotypes, including the calcium-binding proteins calgranulin A and B, calgizzarin, and calmodulin. Spatial distributions for these differentially expressed proteins within the injured regions were confirmed by immunohistochemistry.

Cutaneous wound repair is a complex series of well-orchestrated processes that normally culminates in restoration of tissue integrity. Ideally, the final end product should result in a segment of skin that is fully restored to its former state with identical numbers of skin appendages, comparable tissue elasticity, and acceptable cosmesis. However, the exigencies of wound repair leave much to be desired. Wounds in postnatal humans typically undergo rapid repair that is characterized by extensive fibrosis, contraction, and loss of elasticity. While these

phenomena are useful in helping the organism achieve swift closure, there is wide agreement that the ultimate cutaneous outcome is suboptimal. The MRL/MpJ-Fas^{lpr} (MRL) mouse possesses a remarkable capacity to regenerate full-thickness ear wounds.¹ In contrast to the expected fibrosis and delayed closure, wounded ears of this strain actually regenerate the central cartilage and proceed to complete restoration of the defect.² Similar properties have been described in a cardiac injury model.³ Recent evidence also suggests that these regenerative properties were not present in the body wall skin, as neither incisional nor excisional injuries to the dorsum showed a regenerative phenotype, and wounds healed with the same extent of fibrosis as did two other control strains of mice.⁴ Likewise, another form of cardiac injury did not exhibit scarless healing.⁵ Nevertheless, the existence of an ear model that shows remarkable regenerative properties provides an opportunity to characterize proteins that favor regeneration and restoration of tissue architecture as opposed to normal fibrosis and scarring in response to dermal injury.

Tissue profiling matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS), introduced by Chaurand et al.,⁶ allows the measurement of protein expression patterns from a variety of biological sources, including cells, serum, and tissue sections (reviewed in Chaurand and colleagues^{6,7}). With this method, a tissue section is mounted on a MALDI target plate, matrix solution is manually or robotically deposited directly on specific regions of the tissue section to be “profiled,” and each dried matrix spot is analyzed by a MALDI mass spectrometer. A UV laser is used to desorb and ionize peptides and proteins from these matrix spots. Ionized molecules are accelerated down a flight tube of a time-of-flight (TOF) mass spectrometer and the mass-to-charge (m/z) ratio of each ion is calculated. Data obtained from such an analysis consist of mass spectra in which the peaks correspond to the protonated molecular forms of desorbed peptides or proteins. MALDI MS produces predominantly singly charged protonated molecules, and so the m/z value represents the molecular weight of the protein plus one proton. For proteomic analysis of several homologous regions within the same specimen, multiple matrix spots can be deposited on the tissue surface and the data can be collected as a series of mass spectra. Each mass spectrum contains signals from many hundreds to thousands of proteins and peptides specific to that tissue region.

In the current study, we utilize tissue profiling technology to examine the wound-healing phenomenon to detect candidate proteins that may favor tissue regeneration over fibrosis and scarring in the MRL mouse. Protein profiles were obtained from histologically defined, regenerating regions of MRL full-thickness ear punches and matched regions from the nonregenerative, C57BL/6 control mice. Protein expression at sites of wound repair was profiled at 4 and 7 days postinjury. Spectral analysis revealed distinct differences in the magnitude, temporal sequence, and composition of protein expression patterns between the regenerative and nonregenerative phenotypes. This report features the calcium-binding proteins, calcyclin (S100A6), calgranulin A (S100A8/MRP8), calgranulin B (S100A9/MRP14), calgizzarin (S100A11), calvasculin (S100A4), and calmodulin. Spatial distributions of cellular populations expressing each of these differentially expressed proteins within the injured regions were confirmed by immunohistochemistry.

MATERIALS AND METHODS

Wound induction

All wounding procedures were carried out with the approval of Vanderbilt's Institutional Animal Care and Use Committee. Mice were obtained from Jackson Laboratories (Bar Harbor, ME). The MRL mouse was selected for its documented regenerative capacity.¹ The C57BL/6J strain was selected as the control as it heals ear wounds very slowly and it exhibits scarring and fibrosis in response to injury.⁸ Mice were 8–10 weeks of age at the time of wound induction.

Anesthesia was induced by inhalation, after which two full-thickness excisional defects (2 mm in diameter) were created in each ear with a sterile dermal biopsy punch. Ears were covered with an occlusive dressing (Tegaderm, 3M, St. Paul, MN) to prevent mechanical disruption to the healing tissues due to grooming behavior. Wounds were allowed to heal for 4, 5, or 7 days. At the time of sacrifice, mice were euthanized by CO₂ asphyxiation. The ears were removed, bisected through the wound site, trimmed, and either frozen for MALDI tissue profiling analysis or fixed overnight in 10% neutral-buffered formalin for subsequent infiltration and incorporation into paraffin blocks for immunohistochemical procedures.

Tissue sectioning

Ear punches at both the 4- and 7-day intervals after injury were sectioned (12 μ m thick) with a Leica Jung cryostat (Leica Microsystems AG, Welzlar, Germany) at -15°C . Tissue sections were microscopically examined to ensure that the regenerative bulb (or matched region in the control strain) as well as the distal region of the ear were procured. Sections were transferred to MALDI-compatible conductive glass slides (Delta Technologies Ltd., Stillwater, MN) to aid in deciphering the morphology of each section⁹ and thaw mounted by simply warming the plate. Specimens were maintained at -80°C until further use. Before matrix deposition, sections were dried in a vacuum desiccator at room temperature for at least 30 minutes.

Sample preparation

Matrix (sinapinic acid at 30 mg/mL in a mixture of 50: 50: 0.3 acetonitrile/H₂O/trifluoroacetic acid by volume) was carefully deposited over the MRL-regenerative bulb or the comparable nonregenerative region in the matched control strain. Four mice per strain with two wounds per ear at each timepoint were analyzed, and three sections were examined per wound. For droplet deposition, two 200 nL drops of matrix were manually deposited onto the regenerative bulb of the tissue sections. The first droplet was incubated in a desiccator to near-dryness before the second droplet was deposited. Matrix droplets were then crystallized in a desiccator for approximately 30 minutes.

MALDI MS analyses

Care was taken to maintain the same instrument settings and data processing parameters throughout the study. Mass spectrometric analyses were performed in the positive linear mode at an accelerating potential of +25 kV using a Voyager DE-STR TOF mass spectrometer (Applied Biosystems Inc., Framingham, MA). The instrument is equipped with a 337 nm N₂ laser operating at a 20 Hz repetition rate. One thousand laser shots were acquired per spot on a tissue section. Each mass spectrum was externally calibrated from a protein mixture containing porcine insulin (m/z 5,777.60), bovine cytochrome *c* (m/z 12,232.0), equine apomyoglobin (m/z 16,952.0), and bovine trypsinogen (m/z 23,976.0), and internally calibrated with peaks from murine histones H2B2 (m/z 13,805) and H4 (m/z 11,306). Mass spectra were baseline corrected, Gaussian smoothed, and calibrated using Data Explorer software (Applied Biosystems, Foster City, CA).

Immunohistochemistry

Paraffin-embedded sections (5 μ m) were prepared for immunohistochemical staining. Goat polyclonal anti-human antisera for calcyclin (E-20, 1: 200 dilution) or goat polyclonal anti-human antisera for calgranulin A (C-19, 1: 200 dilution, both purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA), were applied to tissue sections for 60 minutes, followed by incubations with the goat ABC Elite Kit (Vector Laboratories, Burlingame, CA). Rabbit polyclonal anti-human calgizzarin antisera (Proteintech Group Inc, Chicago, IL) at a dilution of 1: 400 or a rabbit polyclonal anti-human calgranulin B (H-90, 1: 50 dilution, Santa Cruz Biotechnology Inc.) were utilized and detected using the rabbit EnVision Plus kit

(Dakocytomation, Carpinteria, CA). A rabbit polyclonal anti-human calvasculin was used at a dilution of 1: 2,500 (Dakocytomation) and detected with the LSAB2 kit (Dakocytomation). Immunoreactivity in this study was visualized using the Nova Red Chromagen (Vector Laboratories).

Statistical analyses

All mass spectra were converted to text files and imported into ProTSDData Version 1.1 (Biodesix Inc., Steamboat Springs, CO) for baseline correction and normalization by total ion current in batch mode. A standard weighted-means-averaging (WMA) algorithm was then applied. The weight value (W) is a statistically derived function that approaches significance as the distance between the means (μ) for each group increases and the SD (σ) decreases using the formula $W = (\mu^1 - \mu^2)/(\sigma^1 + \sigma^2)$. Thus, the most significant protein peaks were then used as the top-weighted data points (m/z intensities).^{10,11} In this way, m/z values were filtered according to the highest weight that best differentiated the MRL vs. control groups. Further filtering was carried out first to exclude values with WMAs < 1.0 (similar in respect to 2σ from the mean control value), and second to exclude mean intensity differences that fell below twofold (cutoff value for tissue profiling, data not published). The filtered values were then used for peak detection, and further evaluated by plotting the entire spectrum in Origin 7.0.

RESULTS

MALDI MS tissue profiles from selected healing regions of control and MRL excisional injuries were acquired, internally calibrated, baseline corrected, and normalized. Figure 1(A) shows a simple schematic describing the tissue profiling methodology used in this study. Frozen tissue specimens were sectioned in a cryostat, transferred to a MALDI target plate, and allowed to dry in a desiccator. MALDI matrix was manually pipetted onto regions within the tissue to be “profiled” and the matrix droplet was allowed to crystallize in a desiccator. In these experiments, matrix was deposited specifically over the expanded bulbous ear wound edge (or control) (Figure 1B and C). The tissue specimen was then inserted into the MALDI TOF mass spectrometer and these areas were irradiated by the laser. Proteins were desorbed/ionized and accelerated propelled down the instrument flight tube, where they were detected sequentially as protonated molecular species $[(M+H)^+]$. As shown in Figure 1D, several hundred proteins were readily detected in this tissue.

Averaged mass spectra representing each mouse strain (control and MRL) were overlaid to compare and identify protein peaks that may discriminate between the control and regenerative phenotypes. Several dozen proteins were observed to be differentially expressed between the two mouse strains as well as across the 4- and 7-day timepoints. Figure 2 shows an expanded view of the averaged mass spectra from m/z 8,625–10,250 (A) and m/z 10,750–13,400 (B). These data also show the relative abundances of several proteins comparing the MRL with the C57BL/6 mouse strain 4 days (Figure 2, upper panels) and 7 days (Figure 2, lower panels) postinjury. As anticipated, most protein signals and their individual relative abundances were quite similar for both mouse strains across time-points. For example, a protein at m/z 12,373, identified previously as a macrophage inhibitory factor,¹² was equally increased following injury of the two strains (Figure 2B). In contrast, a molecule at m/z of 11,306, identified previously as histone H4,¹³ appeared increased in the C57BL/6 mice compared with the MRL. Interestingly, we observed multiple differentially expressed peaks from a well-characterized family of proteins that were previously identified as S100 calcium-binding proteins,^{12–14} including m/z 8,719 (calmodulin fragment), 9,961 (calcyclin), 10,163 (calgranulin A), 10,953 (calgizzarin), 11,639 (calvasculin), and 12,971 (calgranulin B). In the case of calcyclin, no differences were observed in protein signal intensities at either timepoint between ear types. Therefore, calcyclin was not implicated in the process of regeneration in the MRL mouse ear.

Calvasculin was more abundant in the C57BL/6 wounds at both timepoints compared with the MRL. In contrast, expression of the calmodulin fragment appeared to be higher in abundance in the MRL from wounds examined at both 4 and 7 days. Calgranulin A was preferentially enriched in the MRL strain, particularly at day 7. No differences were observed for calgizzarin and calgranulin B at 4 days; however, notable enrichment of both proteins was observed at 7 days in the MRL over C57BL/6.

We focused our immunohistochemical efforts toward providing corroborating observations for each calcium-binding protein (Figure 3). In general, the healing characteristics of the MRL ear wounds were morphologically distinct from the C57BL/6 ear wounds. In the nonregenerative B6 strain, epithelialization of the defect had occurred beneath an inflammatory exudate, and the newly arrived epidermal covering still showed a poorly differentiated epidermis without stratification (Figure 3A, G, and I). In contrast, the newly resurfaced epidermis in the MRL mouse at the same timepoint and in the same region of the wounded ear was highly differentiated with multiple strata (Figure 3B, D, F, H, and J). The thickened epidermal cap was reminiscent of structures observed in regenerating amphibian limbs.¹⁵ All five of the putative calcium-binding proteins from the MALDI spectra were identified in all mouse ears (both regenerating vs. repairing wounds) by IHC.

In agreement with the tissue profiling data, immunoreactive localizations for each of the five calcium-binding proteins were evident in both the scarring and the regenerative phenotype; however, several proteins were notably elevated within the regenerative phenotype. As summarized in Table 1, IHC allowed some preliminary morphological identification of the spatial distributions and cell populations that express each calcium-binding protein. Micrographs in Figure 3B, D, F, H, and J are representative views from the MRL strain. Immunoreactivity to calcyclin was present in poorly differentiated, basal keratinocytes of the epidermis as well as dermal cells (Figure 3A and B). Immunostaining directed against calgranulin A was present in a significant number of large, mononuclear cells adjacent to the injury (Figure 3C and D). The distribution pattern for immunoreactive calgizzarin revealed prominent expression in putative infiltrating mononuclear cells (Figure 3E and F). Calvasculin immunoreactivity was present in perichondrial cells, chondrocytes, monocytes, and occasionally in melanocytes (Figure 3G and H). Immunoreactivity to calgranulin B was widespread and appeared in nearly every cell type at the healing site (Figure 3I and J).

DISCUSSION

Tissue profiling MALDI MS allowed us to acquire highly homologous protein spectra specific to the regenerative (MRL) or nonregenerative (C57BL/6) healing margins of ear punches 4 and 7 days postwound induction. Examination of healing wounds at two different intervals revealed proteomic differences and similarities. The proteomic data showed many differences in protein expression patterns that discriminated between the regenerative and nonregenerative mouse wound, and this report focused on several calcium-binding proteins that were present at both time-points in both mouse strains. Immunohistochemical staining of ear punch sections verified the presence and distribution of proteins that had been identified from MALDI tissue profiling experiments.

Since its introduction, MALDI MS has become an effective tool for discovering tissue-specific proteins from various types of diseases, including cancers of the brain, lung, and several other organs.⁷ For example, in a study of human prostate cancer, tissue profiling revealed overexpression of protein PCa-24 in the cancerous tissue, indicating its potential role as a biomarker.¹⁶ Similar studies have been carried out in azoxymethane-induced colon tumors in mice¹⁷ and biopsies obtained from patients with non-small-cell lung carcinoma (NSCLC)¹⁸ as well as soft tissue sarcoma.¹⁹ Several tumor-specific and tumor grade-specific protein

biomarkers were identified in these studies. In the case of NSCLC, protein profiling allowed classification of lung cancer histologies, predicted survival, and distinguished primary tumors from metastatic tumors of the lung. Tissue profiling has also been used to characterize tissue-specific molecules in a variety of noncancerous pathologies and biological phenomena, including glomerulosclerosis,²⁰ Parkinson's disease,²¹ diabetes,²² and neurological development.²³ Most recently, other proteomic technologies have been applied to the skin and several types of skin conditions.^{24,25}

Tissue profiling MALDI mass spectrometry allowed us to identify a cluster of proteins within the S100 family that are dynamically regulated during the regenerative and scarring processes. The S100 family of proteins presently consists of 21 low-molecular-weight (~9 kDa) proteins that contain two calcium-binding EF-hand motifs. Structurally, these molecules exist intracellularly as antiparallel hetero- and homodimers and interact with target proteins after calcium binding to regulate a variety of cell functions, including chemotaxis, signal transduction, inflammation, and cellular stress.²⁶ In cancer, members of the S100 family members interact with cytoskeletal elements, including microtubules and actin, leading to dysfunction in microtubule assembly and increased cellular motility and invasion.²⁷ Our studies identified a higher abundance of calgranulins A and B in the MRL strain, both of which may be associated with activation and accumulation of granulocytes and activated monocytes/macrophages at inflammatory sites.²⁸ Recent studies have shown that calgranulins A and B are differentially expressed in free-electron laser and scalpel incisions,²⁹ and both molecules are associated with partially differentiated keratinocytes in vitro, suggesting a role for both molecules in the reorganization of the keratin cytoskeleton in the wounded epidermis.³⁰ In addition, a time dependent, increased expression of calgranulin A in fibroblasts upon FGF-2 and IL-1b stimulation, and down-regulation by TGF- β have been observed, supporting a role for calgranulin A in fibroblast differentiation at sites of inflammation and repair.³¹ Over-expression of both calgranulin A and B leads to accelerated healing in several wound models (Dasgupta J, et al., unpublished).

Our data revealed an impressive increase in calgizzarin at day 7 in the MRL mouse, suggesting a role for this molecule in regenerative wound repair. Despite being a key player in tumor transformation and metastasis, the role of calgizzarin in wound repair and regeneration has not been explored extensively. One study detected increased immunostaining patterns in isoproterenol-treated rat hearts, suggesting the possible involvement of calgizzarin after myocardial damage.³²

Calvasculin, also known as fibroblast-specific protein-1 and S100A4,³³ is known for its ability to induce changes in cell shape, particularly in elongating cells.³⁴ Cellular motility, tumor cell invasiveness,³⁵ and enhancement of endothelial cell motility including neovascularization³⁶ have all been ascribed to this protein. In the kidney, this protein is associated with epidermal-mesenchymal transformation and the fibrotic response to TGF- β .³⁷ In wound healing, studies have implicated calvasculin in corneal regeneration in stromal fibroblasts,³⁸ astrocytic response to injury after spinal cord transection^{39,40} and reepithelialization of murine hair follicles after plucking.⁴¹ Our proteomic data showed lower levels of calvasculin in the MRL mouse ear wound compared with the C57BL/6 strain at both time-points. Recently, it was reported that decreased protein levels of calvasculin result in rapid migration of astrocytes into injury gaps of the central nervous system, while infiltrating calvasculin-postive astrocytes fail to close the wound, resulting in scar formation.⁴²

Calmodulin levels were higher in the MRL wound margin. Calmodulin is a ubiquitous intracellular molecule that binds to and regulates numerous protein targets, giving rise to diverse cellular functions, such as inflammation, apoptosis, muscle contraction, intracellular transport, short-term and long-term memory, gene expression, ion channel function, and the

immune response.⁴³ A recent study suggests that calmodulin is intimately involved in fibroblast-mediated collagen lattice contraction, cell migration, focal adhesion formation, and wound contraction, supporting the idea that fibroblast-derived calmodulin is essential for tissue repair.⁴⁴

To our knowledge, this is the first study to use MALDI tissue profiling to explore proteomic differences between the MRL-regenerative mouse strain and its control counterpart. In an earlier work with an off-line MALDI process, termed “surface-enhanced laser desorption and ionization,” or SELDI, proteomic differences were measured between extracts of these mouse models.⁴⁵ In agreement with our studies, calgranulin A was detected as a candidate molecule that might be involved in regenerative repair. In our current study, direct tissue profiling allowed us to mine the proteome of specific regions of the wound site from intact tissue sections, thereby preserving tissue architecture and protein localization. Several hundred proteins were detected from the profiled areas and several dozen differences were detected between the mouse strains at both timepoints. Ongoing work in our laboratory seeks to identify other proteins that are differentially expressed between the mouse models.

Acknowledgments

We are grateful to Nancy Cardwell and Alonda Pollins for their expert assistance in performing the IHC experiments and to Cheryl D. Carlisle for the mouse surgery. This work is supported by the Department of Veterans Affairs (JMD) and NIH grants GM40437 (LBN), P30 AR41943 (SO, JMD, LBN), NIH/NIGMSR01 GM 58008 (RMC), and AG06528 (JMD).

Glossary

B6	C57Bl/6
IHC	Immunohistochemistry
MALDI	Matrix-assisted laser desorption ionization
MRL	MRL/MpJ-Fas ^{lpr}
MS	Mass spectrometry
<i>m/z</i>	Mass-to-charge ratio
TOF	Time-of-flight

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Figure 1.

(A) Basic methodology of preparing tissues for MALDI tissue profiling analysis. Frozen tissue specimens are sectioned in a cryostat (10–12 μm thick), thaw-mounted on a MALDI target plate, and briefly dried in a vacuum desiccator. Serial sections are also prepared for H&E and immunostaining. Matrix droplets ($\sim 200 \text{ nL} \times 2$) are manually deposited onto discrete regions of the tissue and allowed to dry in a vacuum desiccator for at least 30 minutes. The target plate is inserted into a MALDI time-of-flight mass spectrometer and a laser is used to irradiate the matrix droplet. Molecules, mostly proteins, are desorbed/ionized and detected as a function of their time-of-flight where each peak represents one specific protein. The mass-to-charge (m/z) ratio represents the protonated molecular species $[(M+H)^+]$. (B, C) An unstained frozen section of the ears of a healing mouse before and after matrix deposition. The area to the left shows the expanded, bulbous edge of the healing ear wound while the right region of the section shows the morphology of intact tissue. The arrow indicates the central, auricular cartilage. Arrowheads indicate the newly resurfaced epidermal tip. The circular matrix droplet that covers the healing tip of the ear has a diameter of about 1 mm. (D) The cumulative mass spectrum from the MRL mouse ear wound at day 7. The molecular weight range 5,800–70,000 Da is shown. MALDI, matrix-assisted laser desorption ionization.

Figure 2.

Average mass spectra comparing protein profiles between C57BL/6 and MRL mice. (A) The averaged mass spectra of 8,625–10,250 Da. The upper panel compares the 4-day (4D) time point between control and MRL mice and the lower panel compares the 7-day (7D) timepoint between animals. (B) The average mass spectra from 10,750 to 13,400 Da. The upper panel compares the 4D timepoint between control and MRL mice and the lower panel compares the 7D time point between animals. Peak height represents relative protein abundance comparing one specific protein between timepoints and mouse strains.

Figure 3.

Immunohistochemical staining of mouse ears at 5 days after injury. Micrographs in (A, C, E, G, I) are representative views from the C57BL/6 strain. The epithelial cap shows a newly stratified epidermis beneath a scab comprised of inflammatory debris. Micrographs in (B, D, F, H, J) are representative views from the MRL strain. The resurfaced epithelial tip shows a highly differentiated, well-stratified epidermis. (A and B) Confirm the cellular distribution for calcyclin in the healing region of the ear. Immunoreactivity for this protein is quite abundant in the nuclei and the cytoplasm in poorly differentiated keratinocytes of the epidermis. A robust population of dermal cells also expresses this protein. (C and D) Indicate that calgranulin A is restricted to and highly expressed in a substantial subset of large mononuclear cells that are

invading the dermis adjacent to the injury. (E and F) Immunoreactivity for calgizzarin, which is prominent in the nucleus and weakly present in the cytoplasm of the epidermis as well as an infiltrating population of mononuclear cells. (G and H) Confirm immunoreactivity for calvasculin. This protein is expressed both by perichondrial cells that are streaming toward the wounded edge, chondrocytes, and moderate numbers of mononuclear cells. Only an occasional epidermal cell is stained in the undisturbed epidermis and these are presumed to be a population of melanocytes. (I and J) Confirm immunoreactivity for calgranulin B. Numerous cell populations contain this protein. The distribution pattern includes nuclear staining in the epidermis, mild cytoplasmic staining, and over 75% of the dermal cells. Scale bar=220 μm , Inset scale bar=40 μm .

Table 1

Summary of differential expression profiles of calcium-binding proteins between MRL and control mouse strains at 4- and 7-day timepoints as well as cellular localization

Protein	S100 isoform	Day 4 (MRL vs. C57BL/6)	Day 7 (MRL vs. C57BL/6)	Cell type
Calcyclin	S100A6	0	0	Keratinocytes, dermal cells
Calgranulin A	S100A8	+	+++	Monocytes
Calgizzarin	S100A11	0	++	Monocytes
Calvasculin	S100A4	–	–	Perichondrial cells, chondrocytes, monocytes, melanocytes
Calgranulin B	S100A9	0	+++	Large variety
Calmodulin fragment	NA _(a)	+	++	NA _(b)

The S100 isoform is provided in the second column. “0” represents no change in protein expression comparing MRL to control. Levels of increased protein expression comparing the MRL to WT mice are described as “+, ++, or +++” and decreased protein expression is represented by “–.” Specific cell types expressing each molecule are outlined in the fifth column. NA represents (a) no known S100 nomenclature for calmodulin fragment and (b) a lack of an appropriate antibody for IHC analyses. MRL, MRL/MpJ-Fas^{lpr}; IHC, immunohistochemistry.