LYMPHOID TERTIARY AUTO-IMMUNIZATION: THE INTRINSIC CAUSE OF FAILURE

IN CHRONIC REFRACTORY WOUNDS, AND RESTORATION OF WOUND HEALING WITH ANTI-IMMUNE THERAPIES

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Abstract

Wounds due to autoimmune disorders characteristically heal more slowly than other diagnoses. There are also wounds which, even absent overt autoimmune connective tissue diseases, likewise exhibit characteristics, pathological behaviors, and delayed healing trajectories characteristic of autoimmune disorders. These are typically the refractory ulcers which persist for months or years and which defy all efforts and modalities to treat them.

This paper presents histologic, serologic, and clinical data elucidating the pathophysiology of such wounds and wound failure: (1) there is a primary disorder (localized or systemic) of repetitive injury, inflammation, allergy, immunity, infection, or thrombosis; (2) this creates a state of "sustained acute inflammation" in the wound in which the sequential (timewise) phases and anatomical (spatial) strata of the wound lose sequestration and become intermixed; (3) wound intermixing juxtaposes immune antigen processing cells with inflammatory cells, reparative stroma, and their debris, eventually inducing autoimmunization against wound degradation materials; (4) since wound healing is simply the generic stroma reconstituting itself, degradative neo-antigens which induce autoimmunity must of necessity derive from the stroma (angiocytes, fibroblasts, vessels, connective matrix); (5) stromal auto-immunization becomes intrinsic in the wound as dense aggregates of lymphocytes and plasmacytes surround vessels and matrix; (6) these lymphoplasmacytic infiltrates qualify as lymphoid tertiary organs, and once present, proper re-organization of stromal elements within the wound is disrupted or inhibited, manifest clinically as the impaired non-healing wound.

Such patients often have symptoms, signs, and serologies indicative of rheumatoid or lupus-like disorders. These wounds have often failed all other reasonable and appropriate treatments, but they heal when treated with anti-inflammatory and anti-immune drugs (e.g. corticosteroids, azathioprine, 6-mercaptopurine, cyclophosphamide). Rheumatological "collagen-vascular diseases" and the failing lymphoid wound share a common pathway to auto-immunity against connective stroma. As such, lymphoplasmacytic wound failure can be considered a connective tissue disorder with similar treatment methods.

Introduction

Many persistent wounds are due to primary pathologies extrinsic to the wound healing process. In an arterial ulcer, nothing is inherently wrong with the wound healing "machinery". Restore circulation, and the wound heals promptly. Venous ulcers may heal quickly when good compression controls edema and vascular stasis, and pressure ulcers improve when adverse forces are offloaded. However for some wounds, correction of the primary pathology does not restore wound healing. These impaired wounds are stubbornly refractory to most therapies, generally behaving similar to ulcers due to overt auto-immune connective tissue disorders. Indeed, these patients may already have a rheumatoid or lupus diagnosis, but often the primary diagnosis is of a hypercoagulable or other vascular or hemtological micro-occlusive disorder, or else some primary pathology related to allergy or immunity or inflammation for reasons initially independent of the wound. Why do these wounds develop this refractory persistence, and what is the significance of the primary disorders just mentioned?

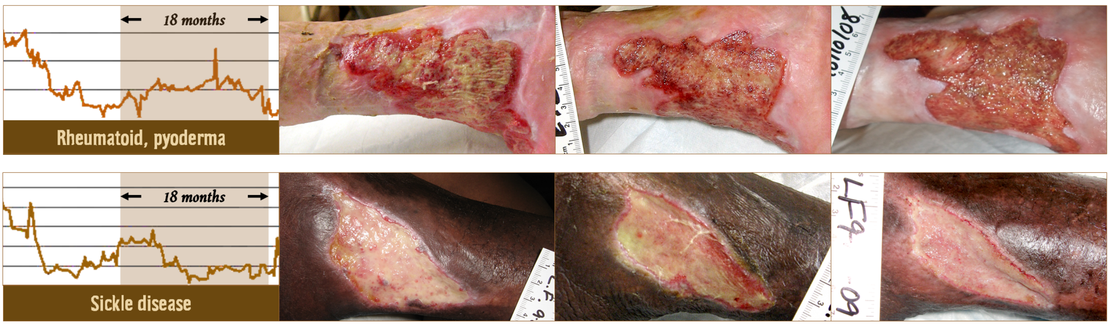
The answer is that under certain circumstances of sustained injury, thrombosis, or inflammation (due to those other primary disorders), wound anatomy and dynamics get "scrambled", and in that persistence and mixing, there is the possibility that neo-antigens from the injured tissue will become the object of lymphoid immunization. Lymphocytes and plasma cells thus induced are targeted against the "wound", i.e. the cells and structures of the generic stroma. Normal wounds have just 2 cell populations - acute inflammation and reparative stroma (angiocytes and fibroblasts). When lymphoid cells appear, this third population is associated with disruption of normal stromal reorganization, i.e. wound healing. Because these inhibitory or disruptive cells are locked to the stromal elements, they become an obligatory element of the tissue, and thus the wounds become persistent and unresponsive to most therapies. Treatments which eliminate the lymphoid third population can restore wound healing dynamics to normal.

This thesis is developed in the next six panels: **(1)** immunopathic ulcers are profiled, including the common association of nuclear auto-antibodies with markers of hypercoagulability; **(2)** the temporal dynamics and spatial anatomy of wounds, and why normally certain cells and events stay sequestered from each other; **(3)** the origins of sustained acute inflammation arising from primary diseases of thrombosis and inflammation, and how this leads to a state of temporal and spatial admixture; **(4)** how anti-nuclear antibodies and auto-immunization against the stroma arises from the state of sustained acute inflammation, the wound transitioning from a 2-population wound (acute inflammation and repair) to a 3-population wound (lymphocytes); **(5)** the pathological effects of lymphoid auto-immunization against the wound and stroma; **(6)** the clinical effects of this state as illustrated by case examples.

1 - AUTOIMMUNE WOUNDS – CLINICAL PROFILE

Some wounds refuse to heal

. . . even when gross pathology & etiology are controlled and acute ulceration & inflammation have subsided.



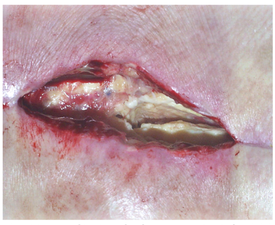
Two examples of the chronic chaotic wound, due to rheumatoid and sickle cell. Over 18 months, there is no net change in wound size nor clinical status, in spite of control of primary disease and numerous attempts to treat.

These behaviors are common in ulcers due to connective tissue disorders (rheumatoid, lupus, etc), but also in ulcers of miscellaneous & uncertain etiology. Treatment & alleviation of recognizable extrinsic factors, such as pressure or arterial insufficiency does not correct the wound as expected if it were due solely to those factors.

Markers of autoimmunopathy are seen in wound patients

. . . with overt CVD-CTD, but also with chronic refractory wounds due to other primary disorders.

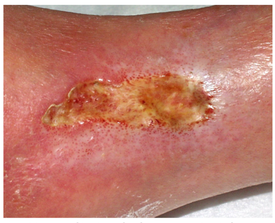
In these examples, some patients had a primary collagen-vascular or connective tissue disorder (CVD-CTD), or else a primary hypercoagulable or other hematological history, or both, or neither. Even with pathognomonic profiles of a specific primary clinical syndrome, laboratory evaluation confirms both states in each patient.



67 F
Rheumatoid Arthritis

F.V Leiden	heteroz	+
protein C	136	H
plasminogen	135	H
fibrinogen	640	H

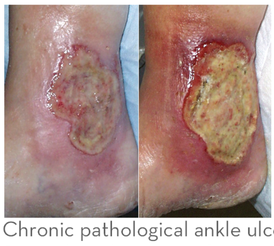
Active clinical rheumatoid. Wound necrosis then impaired healing after laminectomy. Secondary discovery of inborn genetic hypercoagulopathy.



69 F
Rheumatoid Arthritis

F.V Leiden	heteroz	+
protein C	51	L
protein S	52	L

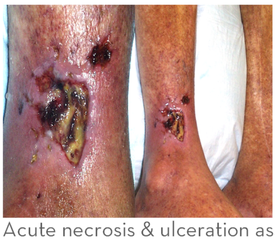
Active clinical rheumatoid. Acute ankle ulceration and impaired healing after total knee arthroplasty. Secondary discovery of inborn genetic hypercoagulopathy.



72 F
Polycythemia Vera

ANA	1:160	H
cardiolipin IgM	80	H
protein S	53	L

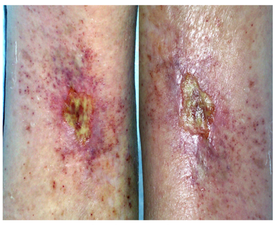
Chronic pathological ankle ulcers associated with P. vera. Secondary discovery of auto-immunopathy (inflammatory) & auto-immune procoagulants (hypercoagulable).



75 M
Anemia / Cythemia

rheumatoid factor	2780	H
cardiolipin IgM	70	H
protein C	65	L
cryoglobulin	pos	+

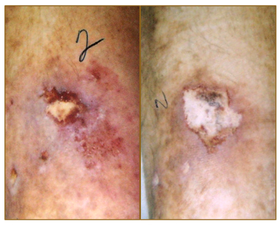
Acute necrosis & ulceration associated with mixed anemia & polycythemia. Secondary discovery of auto-immunopathy, auto-immune procoagulants, and other hypercoag markers.



81 F
No prior diagnosis

rheum factor	27	H
ANA	1:1280	H
lupus anticoag	pos	H
cardiolipin IgM	51	H
protein C	142	H
fibrinogen	429	H
homocysteine	19.3	H

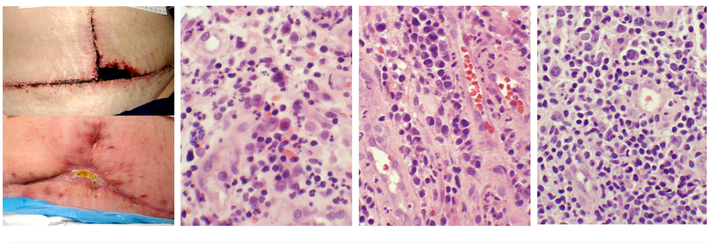
Acute pathological necrosis & ulcer in a patient with no antecedent clinical disease or diagnosis. Laboratory reveals complex mixed autoimmune and hypercoagulable state.



54 M No prior dx

FactorV Leiden	heteroz	+
ANA	1:80-sp	H
lupus anticoag	pos	+
cardiolipin IgA	15	H
cardiolipin IgG	>150	H
cardiolipin IgM	20	H
protein C	60	L
protein S	56	L
homocysteine	14.6	H

Acute necrosis & ulcer in a patient with no prior clinical disease or diagnosis. Laboratory reveals autoimmunopathy & complex hypercoagulopathy (genetic, immune, & metabolic).



53f, lupus. Long history complicated abdominal surgery & wounds; lupus as teenager. New surgery complicated by necrosis then failed wound healing. Edge of wound infiltrated with poly's, nuclear debris, monocytes, & lymphocytes, the recipe for auto-immunization. Elsewhere, lymph & plasmacytes infiltrate along lines of angiogenesis and densely aggregate among old vessels. After prednisone was started, the wound behaved properly and healed.

Autoimmunopathy and lymphoid infiltration in chronic wounds.

What is the connection between auto-immunopathy, connective tissue disorders, hypercoagulability, ulceration and necrosis, lymphoid wound infiltrates, and impaired wound healing? Everything. These events all have an intimate and clinically frustrating cause-and-effect relationship to each other.

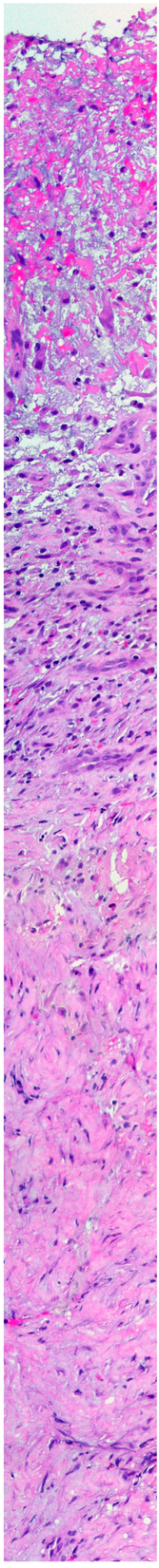
This presentation explains how chronic or repetitive inflammatory or thrombotic states lead to sustained acute inflammation, in turn causing immunization against degradative auto-antigens in the generic stroma (angiocytes, fibroblasts, vessels, matrix). This breeds lymphocytes & plasmacytes (foreign to a normal wound) which perpetuate ulceration or inhibit wound healing. Being locked to the stroma, the lymphocytes become intrinsic to the wound, perpetuating the dynamics which spawned them, holding the wound in a non-healing state. Anti-inflammatory & anti-immune therapies are required to break this cycle of pathology & induce healing.

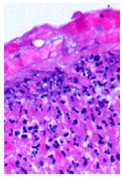
2 - WOUND DYNAMICS – THE HEALTHY WOUND

The healthy wound is a highly structured & organized organ that evolves in space & time.

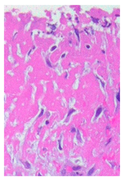
Intrinsic wound cells, are simply angiocytes & fibroblasts, the cells of the generic stroma. Robust cells of long phylogenetic lineage and rare genetic or metabolic pathologies, they fail only when their dynamical interactions are disrupted. This occurs when spatial & temporal wound structure is disorganized and intermixed, leading to abnormal cell populations & interactions. First, observe the normal anatomy & temporal dynamics of a wound.

Sequential structures & events

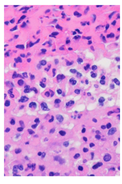




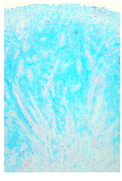
0 - Active injury and inflammation. Inflammation is the response to injury. The wound surface is a plasma protein layer infiltrated with neutrophils.



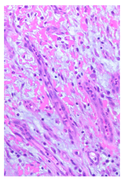
1 - Inflammation subsides. It induces repair, but it is also injurious, and when acute and intense, it suppresses repair. It must subside for repair to proceed.



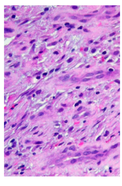
2 - Monocyte-macrophages. Induced by inflammation, these too live in the plasma protein top layer. Their afferent function is phagocytosis and eschar separation. Their efferent function is recruitment of local mesenchyme cells by growth factors.



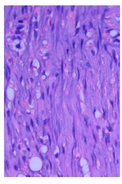
3 - Aminoglycan ground substance. Aminoglycans form under (after) the inflammatory layer, an "ether" in which repair cells will migrate, substance where there is no structure. (Alcian blue stain.)



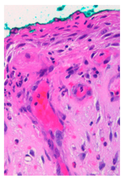
4 - Angiogenesis. Macrophage growth factors stimulate local stromal cells (angiocytes & fibroblasts) to do the repair functions. First is the attraction of angiocytes and the formation of new blood vessels in the aminoglycan layer.



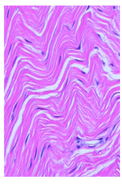
5 - Fibroplasia. Once new vessels restore a suitable environment, fibroblasts start to make durable new body substance, the connective protein matrix. In a healthy wound, inflammatory cells are completely absent at this depth.



6 - Contraction. Once stromal proliferation begins, actual wound closure begins as myofibroblasts contract, reducing the sound surface.

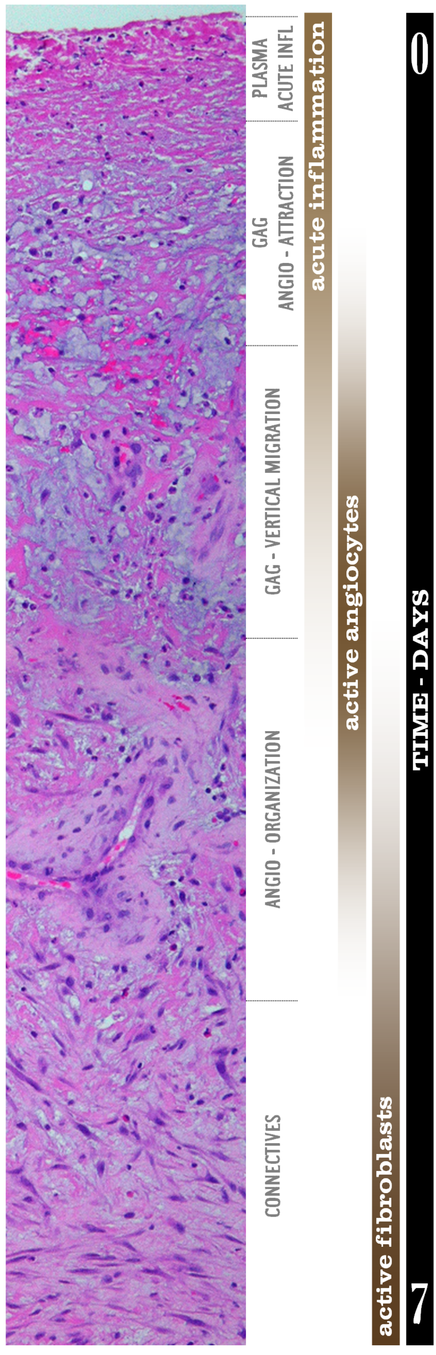


7 - Epithelialization. Epithelium can now grow on the proliferating stroma, and a wound is closed when epithelium is continuous and mesenchyme fully sequestered from the ambient world.

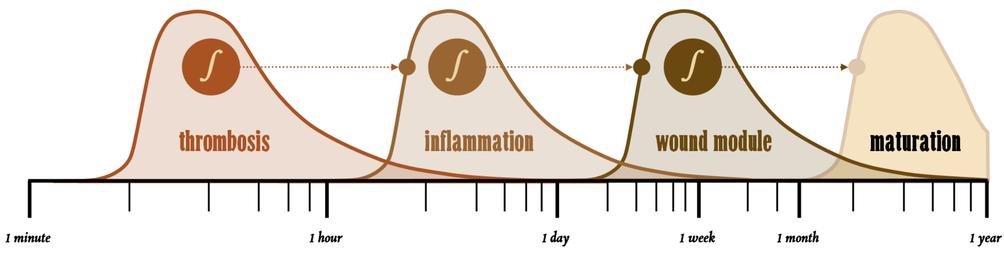


8 - Maturation. Once epithelialized, wounds mature, a gradual process of remodeling young scar back toward the histology of normal stromal tissues such as dermis and fascias.

Temporal & spatial relationships



Temporal structure: Assuming that injury was a one-time incidental event, then inflammation & wound healing events run in sequence, and the cells, structures, and lamina of a normal wound remain separated in time and space. Acute inflammatory cells have little opportunity to meet stromal repair cells. Degradative debris and hence immunological events are minimized.



Normal wound dynamics is a series of "one-shots" of structured events.

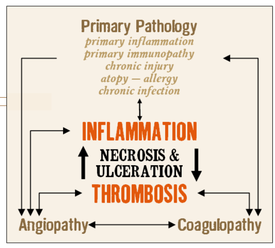
Each major sequential event has a logarithmic (Verhulst) ramp up then decay as it is triggered into action then extinguishes as its purpose concludes. Each cusp is an integrator triggering the downstream event, with each event tailing out as the next event rises. These dynamics are a passive consequence of the inherent closed-loop control within physiological systems. In a healthy wound due to a singular injury, there are no retrograde dynamics, no retriggers, no sustentation, and hence no admixture of events nor their cells & chemicals.

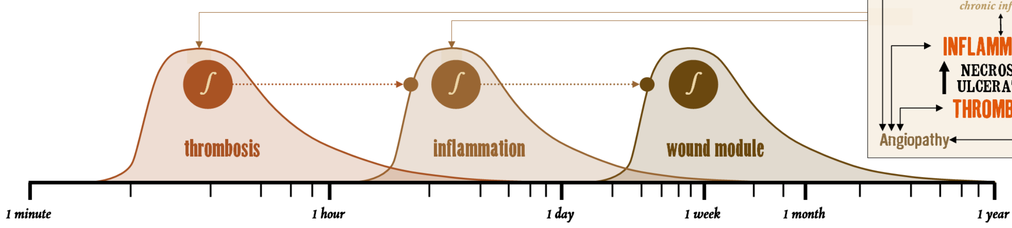
3 - WOUND DYNAMICS – THE SUSTAINED WOUND

Under conditions of repetitive injury or provocation, events are sustained.

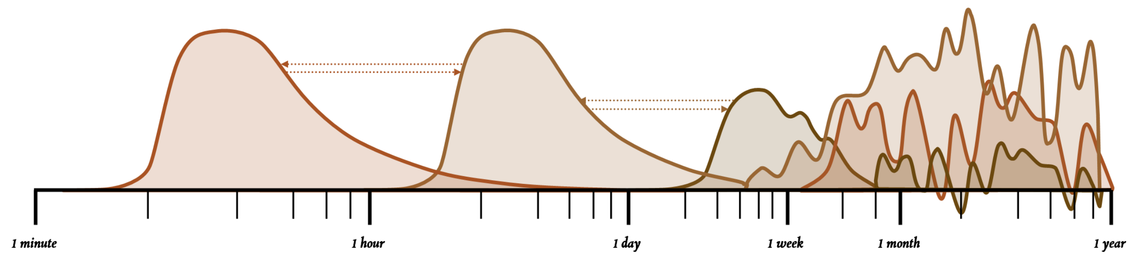
What happens when injury is not a one-time limited event? If repetitive injuries or events occur while prior injury-inflammation-repair events are still active, then there will be retriggering of new events, a superposition of cusps, and eventual self-perpetuation and sustentation of these states. Why? Self-perpetuation has its origins in the crucial inter-relationship between injury, inflammation, and thrombosis.

Thrombosis triggers inflammation, but inflammation also triggers thrombosis and creates more injury. When these events become sustained and mutually inductive, necrosis and ulceration are trapped in the middle. Sustained retriggering of these events can be induced by a variety of primary repetitive pathologies, including primary inflammatory & immunological disorders, trauma, allergy-atopy, chronic infections, and angiopathies & coagulopathies.



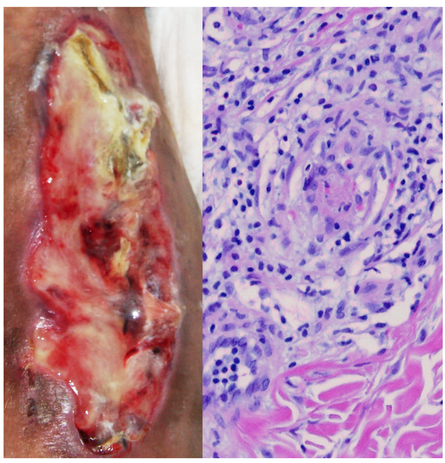


When a new injury or thrombosis or inflammation event is triggered while previous events are still active, then there is a superposition of cusps. As abnormal feedbacks and retriggers occur, the normal healthy one-shot dynamics of proper healing are disrupted. All of these events become erratically persistent and mutually coexistent (technically, this is a non-linear chaotic state).



When that happens, thrombosis, acute inflammation, and wound repair events exist not just concurrently in time, but they also have a chance to migrate and mix within the vertical anatomy of the wound. Cells which ordinarily have little interaction with each other, other than a handshake to pass the baton, become entangled.

74 year old woman with crippling rheumatoid, multiple thrombotic events, & multifocal refractory leg ulcers with thrombo-infarctive & inflammatory-lytic features distinctive of both auto-immune & hypercoagulable states. Histology shows micro-thrombi surrounded by wound healing events (migratory angiocytes) and inflammation. The effect of repetitive sustained injury, thrombosis, and inflammation is to "stir" the mix and perpetuate these events. Observe how a hyper-coagulable state with sustained micro-thrombosis is the primary instigator of this state in this case.

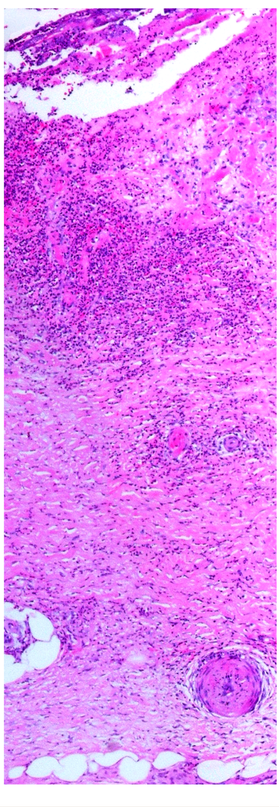


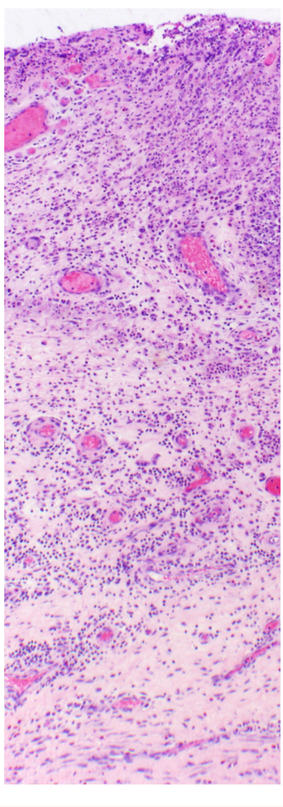
Normal dynamics of injury
The proper response to injury is a set of sequential one-shot events.

Acute Inflammation (AI)
ramps up then decays, occupying a specific time and stratum in the life and anatomy of the of the wound.

Sustained Acute Inflammation
occurs with repetitive & unresolved primary injury causing sustentation of injury and response, and intermix of normal dynamic cusps, cells and structures, the intermix occurring in time and space within the wound

Chronic Inflammation
(next panel) arises only in the perpetuated wound, induced by sustained acute inflammation.





Temporal and spatial admixture in sustained AI:

Left: chronic refractory & progressive leg ulcer due to polyarteritis nodosa. Inflammation is neutrophilic (acute), with leukocytes throughout rather than just at top of the wound, and also concentrated around regenerative new vessels in what would normally be the angio-organization stratum. Remnants of dermis are at bottom, but above there is little organization or distinction of different structures or strata. The persistence and diffuse distribution of these cells & structures mean that wound repair & inflammation are being sustained and perpetuated throughout.

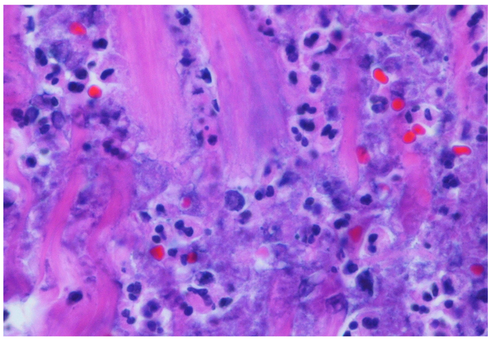
Right: re-ulceration in recently healed skin grafts. This wound is highly admixed. Strata are disrupted. A slight plasma protein top layer & fibrous bottom matrix are present, but in between is homogeneity where there should be defined stratified anatomy. Plasma, aminoglycan & fibrous strata are hard to discriminate. Migratory angiocytes are abundant, but vertical migration zone is gone. New vessels are at all levels, so too neutrophils & monocytes plus a rich infiltrate of lymphocytes, plasma cells, & eosinophils. Indiscriminate admixture causes destruction, inhibition, and disorganization throughout, with no respect to the separation & stratification of time and anatomy that characterize the normal wound.

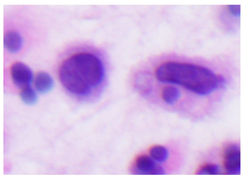
4 - AUTOIMMUNITY & INTRINSIC WOUND FAILURE

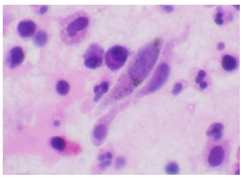
Sustained acute inflammation can lead to lymphoid inflammation

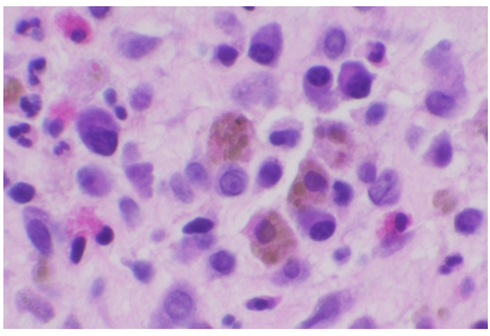
Injury-necrosis-inflammation releases cytoplasmic & nuclear debris – sequestered proteins which, if processed by the lymphoid immune system, would be seen as potential neo-antigens. This does not occur in a healthy wound with sequential short one-shot dynamics and proper vertical stratification, where inflammatory debris and proliferative stromal elements stay mostly separated, and lymphocytes are barely present. However, in the perpetuated wound, with sustained acute inflammation, the load of endocellular material increases, lymphocytes arrive, and cellular intermix leads to conditions of contact and exchange where antigen processing can occur. This can induce a state of auto-immunity directed against cells & structures of these events, the reparative stroma, i.e. the wound. This is the origin of anti-nuclear auto-antibodies, and why CVD-CTD occurs secondary to primary problems of hypercoagulability, chronic infection or allergy, & other sustained pathology.

Endocellular debris and auto-antigens.









Upper: chronic ulcer & progressive necrosis. Note (1) neutrophils & "poly dust" (nuclear debris) plus diffuse basophilia (cellular debris) due to cell lysis, and (2) monocyte-macrophages plus eosinophils & plasmacytes. Nuclear debris is exposed where macrophages and lymphocytes can pick it up. The more material, the longer exposed, the greater the chance of immune recognition & sensitization.

Left: from inflammatory & upper gag layer of a chronic wound, where monocytes & neutrophils dominate. Left is a pre-transformation monocyte; right, a post-transformation macrophage, with a neutrophil nuclear lobe inside, illustrating how inflammatory debris is processed, and the first step of immunization against auto-antigens and the formation of anti-nuclear antibodies.

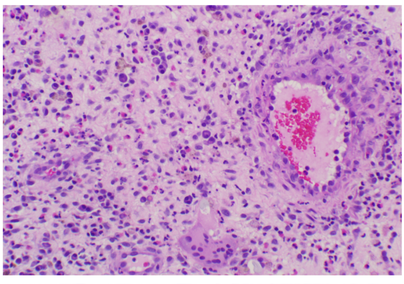
Right: from a chronic progressive refractory ulcer. Note a complex mix of acute (generic reactive) and chronic (immune) inflammation, all within one small field: poly's, nuclear debris, lymphs, plasmacytes, an eosinophil, macrophages, & mesenchymal repair cells (migratory angiocytes). The large central cell, stippled by intracytoplasmic debris, sits next to all of this, closely hugging a plasma cell, showing how debris-processing macrophages will get a chance to exchange something with lymphoid cells.

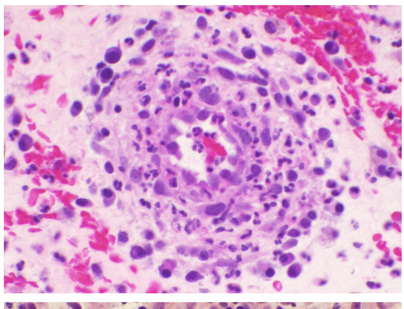
Lower: another refractory ulcer with admixture of multiple populations. Debris laden histiocytes are nestled against lymphocytes & plasma cells against a background of angioid cells, the "perfect storm" for auto-immunization. Risk rises as inflammation is more sustained & cells progressively admix.

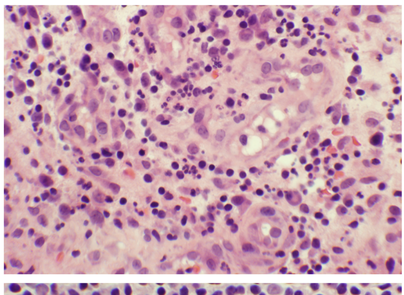
COMMON anti-nuclear & anti-cytoplasmic auto-antibodies

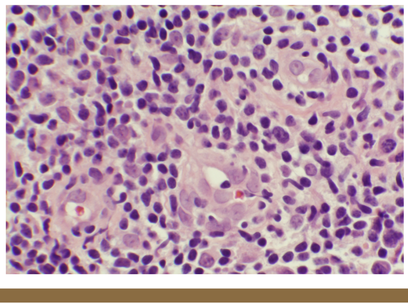
Elevated in the CVD-CTD's, induced by sustained acute inflammation leading to a chronic (lymphoid) inflammatory state

- actin (smooth muscle)
- antinuclear antibodies
- cardiolipin
- centromere
- chromatin
- cyclic citrullinated peptide (CCP)
- dsDNA
- endomysial
- histone
- interleukin-2 receptor
- Jo-1(histidine-tRNA ligase)
- liver kidney microsome (LKM-1)
- mitochondrial
- neutrophil cytoplasmic (ANCA)
- perinuclear ANCA
- ribosomal P
- ribonucleo-protein RNP
- scleroderma (Scl-70)
- sjögren's SS-A Ro
- sjögren's SS-B La
- Sm, Sm-RNP
- tissue transglutaminase (ATA)









Chronic inflammation & the 3-population wound

Shown are 4 chronic active ulcers in patients with prior connective tissue & inflammatory disorders or with a symptom cluster consistent with auto-immune connective tissue disease. In each, wound strata are disorganized, and there is admixture of cells which should never see each other, neither in time (cusps of normal wound healing) nor in space (vertical strata of the wound). In each specimen, there are, side-by-side: (1) ***acute inflammation*** (normal reactive inflammation - neutrophils, neutrophilic nuclear debris, monocyte-macrophages); (2) ***wound module*** of proliferative mesenchymal cells (mainly angiocytes, some early fibroblasts); (3) ***chronic inflammation*** (immunogenic inflammation – lymphocytes, plasmacytes, eosinophils).

Normal healthy wound healing has only two populations – ***acute inflammation*** and ***wound module***. They remain separated in time (by the dynamics of sequential one-shot phases) and in space (by the vertical stratigraphy of the wound). As long as these two populations stay separated, inflammatory cells cannot see stromal cells, and immune sensitization directed against the stroma cannot develop.

The ***3-population wound*** means that "chronic" lymphocytic inflammation has appeared. This occurs only with sustained acute inflammation due to repetitive primary injury, thrombosis, or other ongoing pathology. This in turn leads to temporal chaotic super-position of events and spatial disorg-anization & admixture of cells. This permits acute cells to process matrix debris & present it to immune cells. As antibody producing plasma cells infiltrate the wound, it becomes disorganized, inhibited, and cannot heal, and generalized auto-immune symptoms or markers may become manifest. This state is dynamically "locked in", self perpetuating, and hence stable and difficult to correct.

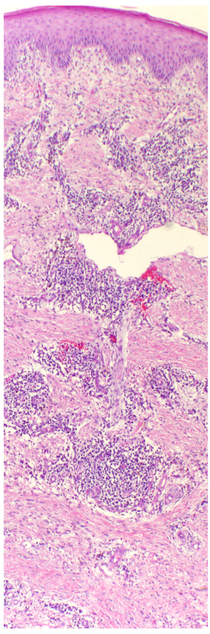
Inflammation & The 3-population Wound

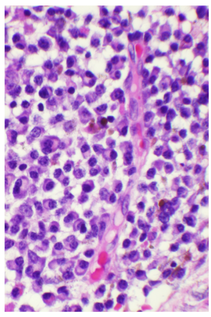
Acute Inflammation
Normal reactive process
Sequence of one-shots
Minimum exposure of sequestered antigen
Minimum macrophage-lymphocyte admixture
No auto-immune sensitization

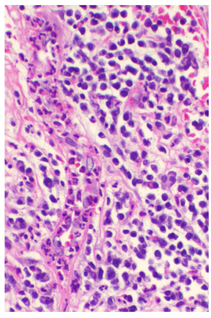
Sustained Acute Inflammation
Repetitive or unresolved primary injury
Not healthy, but can be resolved
Increased load of cellular and nuclear debris
Increased time of exposure
Increased load of reactive cells
Increased chance of lymphocyte appearance
Increased macrophage-lymphocyte admixture
Increased macrophage-lymphocyte interaction

Chronic Inflammation
Immune sensitization has occurred
A new population appears (lymph-plasma)
Perpetuation of injury and altered dynamics
Disorganization & inhibition of wound
Problem is now intrinsic to wound. . .
hence hard to resolve

5 - AUTOIMMUNE INTRINSIC WOUND PATHOLOGY



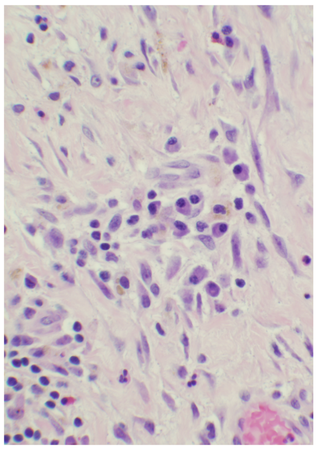




The 3-population lymphoid intrinsic wound

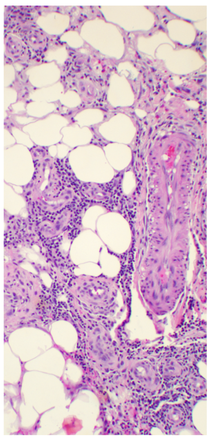
When a wound becomes "intrinsified", self perpetuating because a lymphoid 3rd population has appeared targeted against – and hence coupled to - stromal wound elements, there are several consistent features of these typically refractory and hard-to-heal wounds.

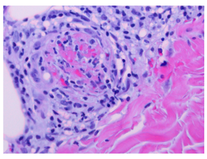
One of the most distinctive features is the affinity of lymphocytes-plasmacytes for angiocytes and blood vessels. In active regions of the wound, planktonic plasmacytes follow migratory angiocytes. In settled areas of the wound and periwound, they form dense aggregates that cluster around vessels.

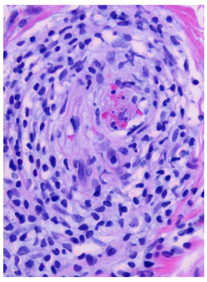


Left: 81f with chronic refractory ankle ulcer after radiation for melanoma. Dense plasmacytes cluster around all blood vessels.

Right: 55f with chronic recurrent abdominal ulcer. Plasmacytes have disrupted the normal vertical migration and re-organization of new vessels.

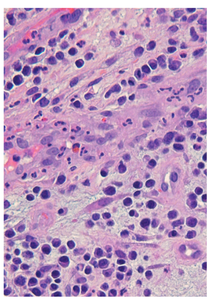




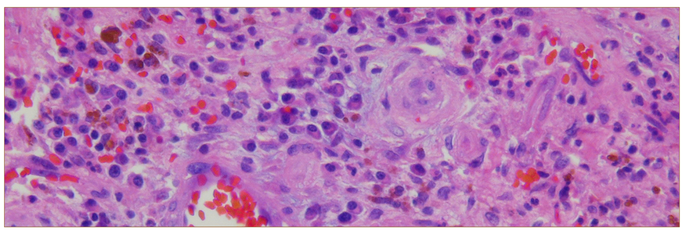


Far left: 65f with hypercoagulability & active rheumatoid. Dense infiltrates surround the vascular loci, mainly lymphocytes with some plasma cells. This refractory ankle ulcer went through cycles of healing then re-infarction & re-ulceration over a year before finally closing and remaining stable.

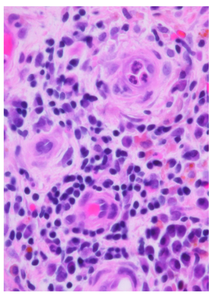
Left: similar 74f with severe rheumatoid, hypercoagulability, & refractory leg ulcers. Vascular lumens are filled with acute & chronic thrombus with neutrophils. Vascular loci are hypertrophied from proper angiogenic activity but also due to inappropriate infiltrates with lymphocytes and plasmacytes. (She healed completely with anti-coagulation, increased anti-inflammatory therapy, and skin reconstruction with regenerative matrices, modalities able to break the sustained acute inflammation and the chronic inflammation.)



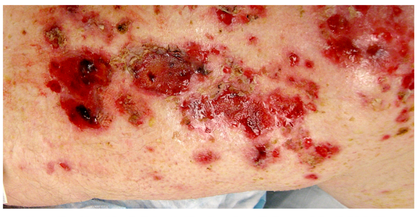
Left: 65m chronic sacral ulcer, remotely due to pressure, but no longer responsive to care. In the angio-attraction & angio-organization gag layer of the wound, plasma cell infiltrates follow the vascular loci and streaming angiocytes.

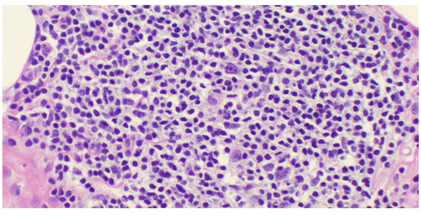


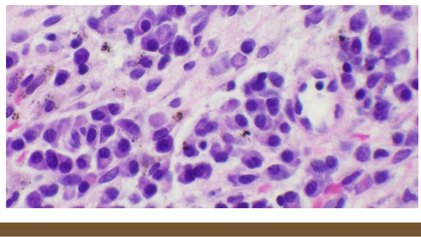
Above: 31 f with thrombosis history, APC resistance, anticardiolipins, secondary auto-immunopathy, and refractory active leg ulcers. There is acute & chronic inflammation, acute & chronic thrombosis, vascular disruption, and peri-vascular plasma cell infiltrates. She too healed after aggressive therapy to break the sustained acute and chronic inflammation.

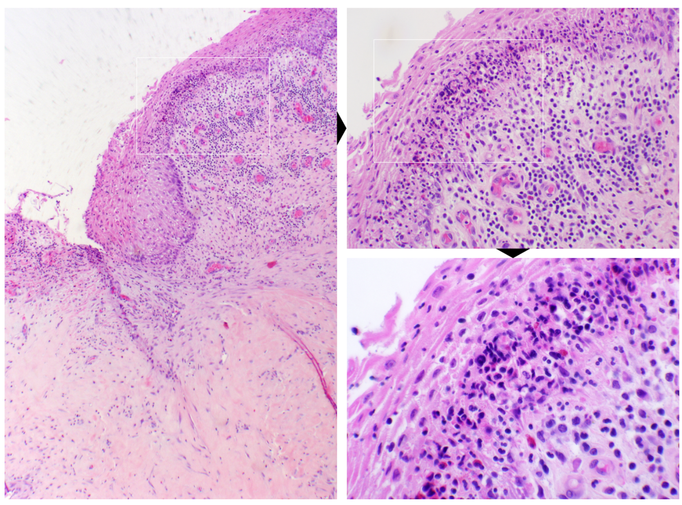


Left: 53m severe rheumatoid. There is admixture of all three populations in this refractory chronic ankle ulcer. Plasmacytes and lymphocytes cluster in and around vessels and angiocytes.









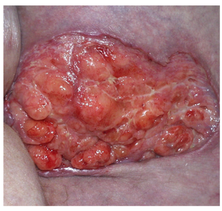
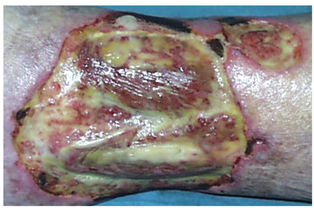
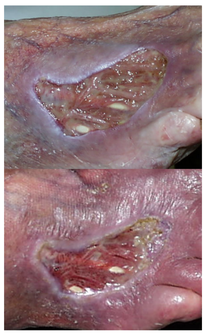
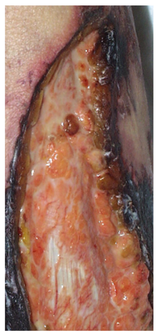
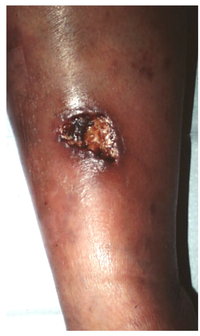
Above: 55f chronic ulcer over a large ventral hernia. Healed regenerative epidermis underwent sudden spontaneous re-ulceration for no apparent reason, controlled with steroids. Biopsy taken during period of acute re-ulceration **Left:** wide view of edge of one of the enlarging lesions. Dermis or scar is inflamed in the upper strata, with a mix of acute & chronic inflammation. The white box zooms on an area of active epidermal lysis. **Right upper:** lower layers of epidermis are eroded by a front of acute leukocytic inflammation, predominated by neutrophils & eosinophils. This activity sits above a zone of lymphocyte infiltrates (and some plasma cells), interspersed in a vascular locus of regenerated immature vessels and migratory angiocytes. The chronic lymphoid infiltrates would seem to have triggered the acute neutrophilic destructive event (**right lower**).

Left: 66m with chronic refractory ulcerative dermatosis (thigh is shown). Lympho-plasmacyte infiltrates were typically around all blood vessels. The lymphoid aggregates do not have features of germinal centers (secondary lymphoid organs), but their micro-architecture and clinical pathology qualify them as true lymphoid tertiary organs.

6 – CASE STUDIES

Effects of auto-immune disorders – Arrested wound module

Auto-immune inhibition or predation against the connective tissues, CVD-CTD, inhibits wound healing because wound repair is simply the stromal connective tissues self re-assembling. Here are examples of complete arrest of the normal proliferative wound module, no healing whatsoever after prolonged periods of observation & care.

Above left: 44f, rheumatoid, buttock ulcer following minor injury 6 months ago. With only a slight blush of angiogenesis, all fat lobules maintain their native anatomy and textures, as though, if normal, it had been created just 3 or 4 days ago.

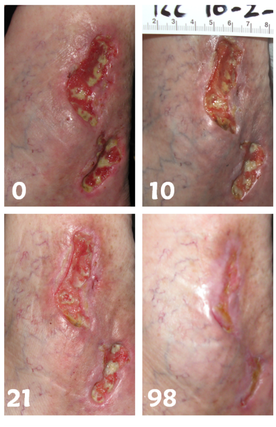
Above right: 73f, prototypical rheumatoid ulcer of ankle. Present 4 years, there is zero wound proliferation – native anatomy shows as if the wound was excised just yesterday. **Center:** 83f, rheumatoid ulcer, dorsal foot. Over several weeks of care, areolar fascias and tendon sheaths are still visible, **Center right:** 35f, acute lupus & hypercoagulable state, with multi organ failure & skin infarcts – no wound module elements after 3 months, with normal thigh fascias still visible. **Right:** 60f, poly-myositis, minor leg injury 4 months prior. Wound module events are so impaired that even eschar is not fully separated yet.

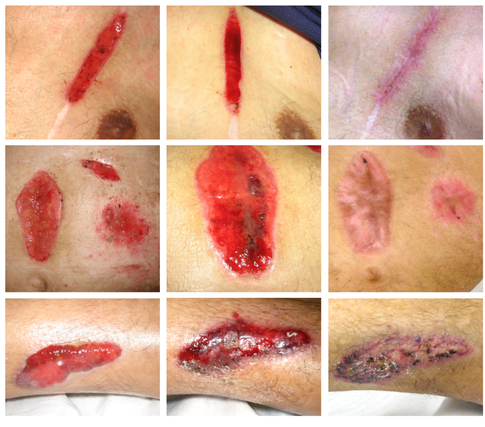
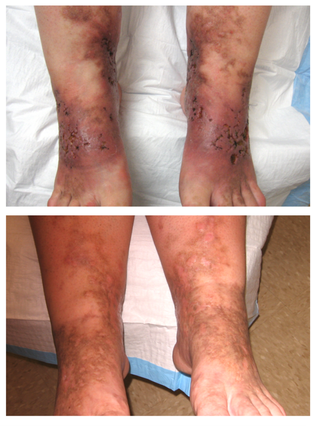
Treatment with anti-inflammatory & anti-immune therapies

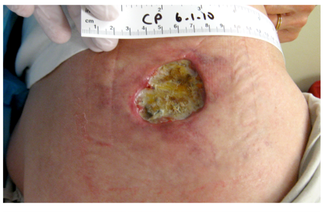
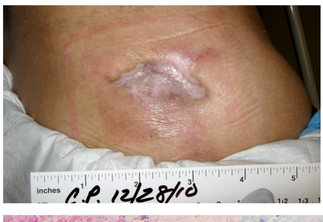
Left: 71m rheumatoid x20 years, poorly treated & uncontrolled, ankle ulcers. Daily care has kept them hygienic but unchanged in 2 years. Intralesional triamcinolone was injected, and patient was started on **prednisone**. At 10 days, pain & gross inflammation gone. At 21 days, healing and area reduction are overt; at 98 days nearly all healed.

Center: 35m, many ulcers trunk & extremities, bullous pemphigoid >> refractory chronic wounds. Histology: acute & chronic inflammation, plasmacytes & eosinophils, impaired wound module lacking fibroplasia. Simple topical care ineffective. Prednisone resolved many small lesions, but not larger ones, so **azathioprine** was started. ***Upper*** row is left pectoral; ***middle*** is abdomen; ***lower*** is leg. ***Left*** column is after initial non-specific care; ***center*** is after 3 months of steroids; ***right*** is 7 months into treatment, 2 months after starting azathioprine - healed.

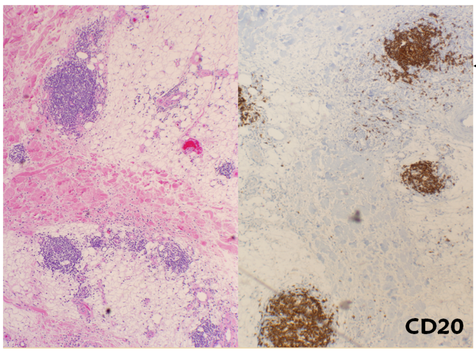
Right: 25f, hypercoagulable (primary) & immune markers. Multifocal vascular stasis, skin infarcts, ulcers. For 2 years, warfarin kept disease quiet and wounds healed, but inconsistently, and then disease accelerated. Steroids were helpful, but not curative. Histology showed perivascular lymphoplasmacyte infiltrates. Cyclophosphamide was started, & she promptly healed. Top, latter stages of active disease unresponsive to prior therapies. Bottom, a few months into **cyclophosphamide**, the legs are healed, inflammatory changes & stasis are gone.

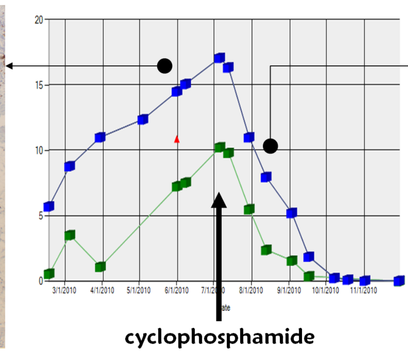


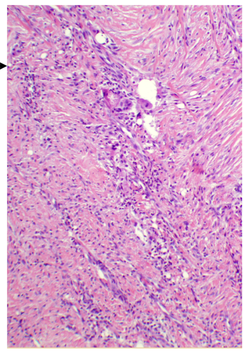



Case: 44f with chronic calcifying panniculitis of the right hip with progressive ulceration. This is a syndromic and usually progressive panniculopathy generally affecting pelvis & thighs, and seen with the connective tissue disorders. The photos show the lesion before definitive therapy, then healed 6 months later. The wound exhibited pathergy, with progressive necrosis with each attempt at debridement. There was no response to intralesional nor oral steroids nor to any topical care. The graphs document progressive increases in wound volume (green) & area (blue) in spite of care. Histology confirmed lympho-plasmacyte infiltrates throughout the vascular locus. **CD20** stain confirmed these as committed antibody producing plasmacytes. Cyclophosphamide was started as the most direct way to control lymphocytes (dose = 1 mg/kg/day). Wound improvements were immediate. Biopsy at 6 weeks showed restoration of normal wound and vessel features without the plasmacytes. The wound was healed within 4 months. After another 4 months of the wound & primary panniculitis remaining stable, the drug was tapered and withdrawn.





cyclophosphamide


EPILOGUE: Some of the most refractory difficult to heal wounds are those related to auto-immune CVD-CTD disorders, not surprising since the wound is nothing more than the connective stroma mending itself after injury. Stromal auto-immunization is apt to occur from disorders which perpetuate injury, thrombosis, and acute inflammation, leading to ***sustained acute inflammation*** which then induces ***chronic lymphoid inflammation***. Once the wound is auto-immunized, lymphoid cells directed against stromal elements induce ulceration and inhibit healing. Treating the auto-immune state and eliminating the lymphocytes can induce wound healing.

Key Points and Summary

The normal 2-population wound. The normal response to injury is the integrated process of inflammation then wound healing. When healthy, the process depends on two cell populations, (1) ***acute inflammation*** with poly- and mononuclear leukocytes, and then (2) the ***wound module*** of proliferative repair, the angiocytes and fibroblasts of the generic stroma reassembling themselves.

Dynamics of the normal wound. Healthy healing has several phases – injury, thrombosis, inflammation, proliferation, then maturation – which are dynamical one-shots. Each cusp rises, completes its task, then subsides. Each triggers its downstream event, but there is little timewise overlap between phases, nor do their cells overlap much in the vertical strata of the wound.

Dynamics of the perpetuated wound. There is an intimate interconnection between injury-inflammation-thrombosis, each with potential to induce or trigger the other two. In healthy wounds, the sequential dynamics have no retriggering nor sustentation of prior phases. However, in conditions of sustained or renewed injury-thrombosis-inflammation, the wound enters a chaotic attractor, ***sustained acute inflammation***, where these events are perpetuated. This is inhibitory to wound healing, and it causes timewise & spatial mixing of wound cells & structures.

The causes of sustained acute inflammation. Any process or pathology which independently triggers thrombosis, inflammation, or injury & necrosis has the potential to perpetuate or sustain acute inflammation and retard the wound. This includes hypercoagulable and micro-occlusive disorders, primary inflammatory and immune disorders, chronic allergy-atopy, and chronic infections.

Auto-immunization. In conditions of sustained acute inflammation, debris increases, exposing stromal auto-antigens from angiocytes, fibroblasts, vessels, & connective matrix. Cell intermix results in opportunities for macrophages to find and process neo-antigens, then present to lymphoid cells. The ultimate event is that sensitized lymphocytes breed antibody producing plasmacytes (foreign to a normal wound) which cluster around the mesenchymal structures that sourced the antigens. Persistent lymphoid aggregates qualify as ***lymphoid tertiary organs***. The appearance of lymphoid cells and aggregates creates the ***3-population wound*** which perpetuates ulceration and inhibits wound healing.

Auto-immunization. This process of stromal auto-immunization is the origin of the collagen-vascular connective-tissue disorders. Since wound healing is simply the process of stromal reorganization after injury, any immunopathic predation against the process is identical to what happens in the CVD-CTD diseases. Because lymphoid infiltrates in the wound are locked to the stroma, the lymphocytes have become an intrinsic part of the wound, perpetuating the dynamics which spawned them, holding the wound in a non-healing state. Anti-inflammatory & anti-immune therapies are required to break this cycle of pathology & induce healing. These wounds occur in patients with primary histories of CVD-CTD, but also primary histories of hypercoagulopathy and related disorders which induce sustained acute inflammation and thereby induce auto-immunization.

Expanded information and resources on this subject are at:

www.arimedica.com

This subject is presented in detail in the following set of notes:

The Physics and Pathology of Wounds - Part 1
The Wound as a System and a Controlled Machine
The wound module, the wound control loop, wound pathology, and the basic dynamics of healthy and impaired wounds.

The Physics and Pathology of Wounds - Part 2
Auto-Immunopathy and the Intrinsic Disease of Wound Healing
The cellular & histopathological basis of intrinsic wound failure & wound chronicity: chronic inflammation, wound autoimmunopathy, & the 3-population wound.

The Physics and Pathology of Wounds - Part 3
Chronicity and the Physics of Wound Failure
The physics of wound failure & chronicity: N-body dynamics & chaos, population logistics, cellular automata & self-organization.

(Not) Atypical Wounds
Autoimmunopathy and Connective Tissue Disorders:
The True Intrinsic Diseases of Wound Healing
Prelude to the above, with focus on the pathology and clinical aspects of immunopathic and coagulopathic ulcers

Current links to these and related materials are at:

http://www.arimedica.com/presentations.htm

http://www.arimedica.com/content/arimedica_wpp-1_wound%20control_gottlieb-me_maui-2010-0222_annotated.pdf

http://www.arimedica.com/content/arimedica_wpp-2_autoimmune%20&%20intrinsic_gottlieb-me_maui-2010-0222_annotated.pdf

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