LA RISPOSTA INFIAMMATORIA CRONICA ED I MECCANISMI MOLECOLARI DEL RIPARO

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Il materiale presente in questo documento viene distribuito solamente per uso interno ed esclusivamente a scopo didattico.
Ever since the initial descriptions of the cardinal characteristics of inflammation – rubor, dolor, calor, and tumour – by the first-century Roman medical writer Aulus Cornelius Celsus, physicians and scientists have attempted to unravel the mysteries behind the human body's response to injury. Recent estimates suggest that chronic inflammatory and fibrotic disorders are responsible for $142 billion in annual United States healthcare costs alone. As a means to begin addressing some of these deficiencies (and many others) in knowledge, the 2013 Annual Review Issue on Inflammation, Wound Repair, and Fibrosis was born.
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**INFLAMMATION, WOUND REPAIR, AND FIBROSIS:** REASSESSING THE SPECTRUM OF TISSUE INJURY AND RESOLUTION

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LA RISPOSTA INFIAMMATORIA CRONICA E LE PATOLOGIE AD ESSA ASSOCIATE
INFIAMMAZIONE CRONICA

Infiammazione di durata prolungata in cui procedono contemporaneamente l’infiammazione attiva, la distruzione di tessuto e i tentativi di riparazione:
- caratterizzata prevalentemente da una risposta cellulare (macrofagi) e da scarsi fenomeni vascolari;
- può rappresentare l’evoluzione di un processo infiammatorio acuto o insorgere in seguito ad una risposta immune cellulo-mediata.

Fattori predisponenti includono stimoli lesivi persistenti e malattie autoimmuni

La guarigione di un’infiammazione cronica avviene sempre per riparazione!
LA NON RISOLUZIONE DELL’INFIAMMAZIONE ACUTA PORTA ALL'INFIAMMAZIONE CRONICA!

PROSTAGLANDINIS, LEUKOTRIENES

COMPLET Resolution

COMPLETE Resolution

Abscess/Scar formation

CHRONIC Inflammation

Time

Tissue injury, Allergen, Microbe

Acute inflammation

Prostaglandins, Leukotrienes

Lipoxins, Resolvins, Protectins

IL1RA

IL10

TGF beta

Maresins
**ACUTE INFLAMMATION**
- Vascular changes
- Neutrophil recruitment
- Mediators

**RESOLUTION**
- Clearance of injurious stimuli
- Clearance of mediators and acute inflammatory cells
- Replacement of injured cells
- Normal function

**FIBROSIS**
- Loss of function

**CHRONIC INFLAMMATION**
- Angiogenesis
- Mononuclear cell infiltrate
- Fibrosis (scar)

**INJURY**
- Infarction
- Bacterial infections
- Toxins
- Trauma

- Viral infections
- Chronic infections
- Persistent injury
- Autoimmune diseases

*INFIAMMAZIONE CRONICA PORTA ALLA RIPARO o ALLA FIBROSI!*
Uno dei fenomeni fondamentali dell'inflamazione cronica è rappresentato dalla continua infiltrazione di macrofagi che derivano dai monociti circolanti e ....
I MONOCITI/MACROFAGI NELL'INFAMMAZIONE CRONICA POSSEONO ESSERE ATTIVATI DA DUE STIMOLI PRINCIPALI!

1. **STIMOLI NON IMMUNOLOGICI**
   - Corpi Estranei/LPS=lipopolisaccaride
   - MONOCITI/MACROFAGI PRODUCONO TNF-α!
   - FAGOCITOSI/IPERATTIVAZIONE/FUSIONE

2. **STIMOLI IMMUNOLOGICI**
   - Citochine e fattori prodotti da varie sottopopolazioni di linfociti T helper (IFN, IL4, IL-17, etc.)
   - MONOCITI/MACROFAGI PRODUCONO TNF-α!
   - FAGOCITOSI/IPERATTIVAZIONE/FUSIONE/RECLUTAMENTO FIBROBLASTI/FIBROSI
I PRINCIPALI MONOCITI/MACROFAGI ATTIVATI DA STIMOLI IMMUNOLOGICI: **M1 e M2**!
Microbes, IFN-γ
Classically activated macrophage (M1)
Alternatively activated macrophage (M2)
IL-13, IL-4
ROS, NO, lysosomal enzymes
IL-1, IL-12, IL-23, chemokines
Microbicidal actions: phagocytosis and killing of many bacteria and fungi
Inflammation
IL-10, TGF-β
Growth factors, TGF-β
Tissue repair, fibrosis
Anti-inflammatory effects
IL-10, TGF-β
Growth factors, TGF-β
Tissue repair, fibrosis
Anti-inflammatory effects

LE FUNZIONI E GLI EVENTI MEDIATI DAI MACROFAGI M1 e M2!

IPERATTIVAZIONE/RECLUTAMENTO FIBROBLASTI/FIBROSI
I LINFOCITI Th1 ORCHESTRANO L’INFIAMMAZIONE CRONICA PERCHE’ RECLUTANO I MONOCITI/MACROFAGI M1!
I linfociti Th2 possono mediare reazioni di infiammazione cronica perché reclutano i monociti/macrofagi M2 e gli eosinofili!!!
Anche i linfociti Th7 possono mediare infiammazioni croniche perché reclutano ed attivano monociti/macrofagi ed i neutrofili!

Psoriasi, Crohn’s disease (CD), etc.
AD ALCUNE REAZIONI INFIAMMATORIE CRONICHE PARTECIPANO LINFOCITI T CD8 CITOTOSSICI!

Diagram:
- CTL
- Cell-associated antigen
- Cytotoxicity
- Contact dermatitis
I monociti/macrofagi attivati da stimoli immunologici (principalmente gli M2) possono portare alla fibrosi, tipica lesione patognomonica dell'inflammazione cronica diffusa!
I monociti/macrocaghi attivati da stimoli non immunologici ed immunologici (principalmente gli M1) possono portare alla formazione dei granulomi: le lesioni patognomoniche dell'inflammazione cronica!
NELLE REAZIONI INFIAMMATORIE CRONICHE
GRANULOMATOSE INDOTTE DA STIMOLI IMMUNOLOGICI
i LINFOCITI ed i MACROFAGI (soprattutto gli M1) si
attivano a vicenda…

[Diagram]

- Linfocita
- Linfocita attivato
- IL-1, TNF
- Macrofago attivato
- Presentazione dell'antigene ai linfociti T
- Altri mediatori dell'inflamazione
- IFN-γ
- TNF-α
E, COME NEI GRANULOMI NON IMMUNOLOGICI, I MACROFAGI IPERATTIVATI SI FONDONO!

MACROFAGI

CELLULE EPITELIODI

CELLULE GIGANTI POLINUCLEATE (Cellule di Langans)
Il GRANULOMA è l'ultimo processo di contenimento di materiale estraneo inerte o biologico: si chiama reazione del "WALLING OFF"!
MECCANISMI DI FORMAZIONE DEL GRANULOMA!!!

DANNO
Batterio (ad es. Mycobacterium tuberculosis)
Fungo (ad es. Histoplasma capsulatum)
Particella estranea (ad es. punti di sutura)

Incapacità di digerire l'agente stimolante

Insuccesso nella risposta infiammatoria acuta

Persistenza dell'agente patogeno

Risposta immune cellulo-mediata

Sequestro entro i macrofagi

Reclutamento di macrofagi, con formazione di cellule epiteliocidi e cellule giganti

GRANULOMA
Figura 3-26. Infiammazione cronica del polmone, nella figura sono evidenti le tre tipiche caratteristiche istologiche: (1) raccolta di cellule dell’infiammazione cronica (*), (2) distruzione del parenchima (gli alveoli normali sono sostituiti da lacune delimitate da epiteli cubico, punte di freccia), e (3) sostituzione con tessuto connettivo (fibrosi, frecce).
LE COMPONENTI PRINCIPALI DEI GRANULOMI NON IMMUNOLOGICI DA CORPI ESTRANEI!

MACROFAGI

- CELLULE EPITELIODI
- CELLULE GIGANTI POLINUCLEATE

FIBROBLASTI

Cellule di Langans
**IL GRANULOMA DA CORPO ESTRANEO**!

<table>
<thead>
<tr>
<th>Eziologia</th>
<th>Caratteristiche istologiche e manifestazioni cliniche</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sono provocati da materiali provenienti dal regno vegetale, animale e minerale o ottenuti per sintesi, tutti caratterizzati da scarsa solubilità e digeribilità, penetrati nell'organismo per via transcutanea o inalatoria. Tra quelli provocati dalla penetrazione per via transcutanea si ricordano a modo di esempio, schegge di legno, spine vegetali e di pesci, aculei di riccio di mare, frammenti di proiettile, fibre tessili, talco, olio (oleoma), paraffina (paraffinoma), materiali da sutura (catcut), metalli, silicone, etc., carbone (come può avvenire nei tatuaggi)</td>
<td>Persistono fino a quando il materiale estraneo non viene eliminato o tolto avendo scarsissima tendenza alla risoluzione spontanea ad eccezione di quelli formatisi per inoculazioni di olio, usato come veicolo per vari farmaci. Il granuloma si presenta istologicamente con caratteristiche sovrapponibili, qualunque sia l'agente eziologico. Esso è formato da una grossa cellula gigante, fornita di molti nuclei distribuiti irregolarmente nel citoplasma, situata al centro e circondata da cellule epitelioidi</td>
</tr>
</tbody>
</table>

Il granuloma contiene macrofagi e cellule giganti derivate da macrofagi. I linfociti sono assenti.
Granuloma apicale dentale

Granuloma da piercing

Xantoma-granuloma lipofagico

Granuloma da corpo estraneo (filo di sutura)

Lesione reattiva simil-tumorale caratterizzata dalla presenza a livello cutaneo di una papula, nodulo o placca, di colore bianco-giallastro dovuto all’accumulo di colesterolo.
LE COMPONENTI PRINCIPALI DEI GRANULOMI DI TIPO IMMUNOLOGICO!

MACROFAGI (soprattutto M1)

CELLULE EPITELIODI

CELLULE GIGANTI POLINUCLEATE
(Cellule di Langans)

LINFOCITI T

PLASMACELLULE

FIBROBLASTI
ALCUNI GRANULOMI DI TIPO IMMUNOLOGICO!

<table>
<thead>
<tr>
<th>MALATTIA</th>
<th>CAUSA</th>
<th>REAZIONE TESSUTALE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Tubercolo non caseoso (prototipo di granuloma): focus di cellule epitelioidi, circondato da fibroblasti, linfociti, istiociti, talvolta cellule giganti tipo Langhans; tubercolo con necrosi caseosa: presenza di detriti granulari amorfi centrali, perdita di ogni dettaglio cellulare; presenza di bacilli acido resistenti</td>
</tr>
<tr>
<td>Lebbra</td>
<td><em>Mycobacterium leprae</em></td>
<td>Presenza di bacilli acido resistenti nei macrofagi; granulomi a cellule epitelioidi</td>
</tr>
<tr>
<td>Sifilide</td>
<td><em>Treponema pallidum</em></td>
<td>Gomma: lesione microscopica o macroscopica, con palizzate di istiociti; infiltrato di plasmacellule; le cellule al centro sono necrotiche, non viene perduta l’architettura cellulare</td>
</tr>
<tr>
<td>Malattia da graffio di gatto</td>
<td>Bacilli Gram-negativi</td>
<td>Granuloma stellato o circolare contenente detriti granulari centrali e neutrofili; rare le cellule giganti</td>
</tr>
</tbody>
</table>

Histoplasmosi  Histoplasma capsulatum  Granuloma that contains a fungus called Histoplasma capsulatum!
Un tipico granuloma immunologico è quello che si forma nella Tubercolosi!

**Figura 4.20 Un granuloma tubercolare.**

In (a) è mostrata, in maniera schematica, la struttura tipica di un tubercolo. Un’area centrale di necrosi caseosa amorfa è circondata da una zona di macrofagi attivati, in cui sono presenti macrofagi multinucleati (cellule giganti di Langhans). Ci sono strati esterni di linfociti e fibroblasti. La fotografia a piccolo ingrandimento (b) mostra un tubercolo del polmone, in cui sono riconoscibili le caratteristiche illustrate in (a). La microfotografia a medio ingrandimento (c) mostra in maggior dettaglio il margin del tubercolo.
SCHEMA GENERALE DELL’ INFEZIONE TUBERCOLOSICA…
.....dove il Mycobacterium tuberculosis viene captato dai macrofagi alveolari ma resiste alla loro azione distruttiva e…….

**M. tuberculosis evades phagocytic death!**
- arrest of phagosome maturation
- block of phagosome/lysosome fusion
CD4+ T, CD8+ T, NK/T, \( \gamma/\delta \) T cells:
- killing of infected macrophage (perforin, granulysin)
- cytokine-mediated activation of infected macrophage
….ma sono soprattutto i Macrofagi attivati dall’ IFN gamma prodotto dai Th1 a contenere l’ infezione tubercolare….

(T cell-mediated activation of macrophages is necessary for the containment of *M. tuberculosis* infection!)

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**Infected macrophage**
- lysosome
- mycobacterium
- antigen

**Activated infected macrophage**
- T<sub>H1</sub>

**Graph**
- Survival, %
- Days after BCG infection

*Figure 1-26 Immunobiology, 6/e. © Garland Science 2005*
Il ruolo del TNF nel granuloma tubercolare

A. TNF derivato dai macrofagi infettati richiama cellule dal sangue nel sito di infezione

B. TNF e IFN-γ attivano i macrofagi che si organizzano a formare il granuloma

C. Il TNF è necessario per mantenere il granuloma e contenere l’infezione tubercolare

Ehlers S.
Why does tumor necrosis factor targeted therapy reactivate tuberculosis?
…ed ad essere continuamente attivati fino alla formazione del granuloma!!!
The granulomatous reaction to *M. tuberculosis* infection: IL GRANULOMA TUBERCOLARE
Main features of tuberculosis: from infection to host defence!

Cytokine-mediated activation of macrophages is necessary for the containment of *M. tuberculosis* infection!

Different *MTB* cell wall components are recognized by different PRR!
I *Mycobacteria tuberculosis*
SONO ATTIVI NEL GRANULOMA ....
DOVE PERSISTONO
Recently, it has underlined the DC–T cell cross-talk within chronic granulomas!

The role of dendritic cells in mycobacterium-induced granulomas

Immunology Letters, Volume 130, Issues 1–2, 4 May 2010, Pages 26–31
Heidi A. Schreiber, Matyas Sandor

(A) DCs with stimulatory phenotype, high expression of MHCII, CD40, CD86, CD80, and secreting IL-12, are likely to support a Th1-IFNγ producing T cell phenotype. This DC phenotype will result in pathogen killing within macrophages through the secretion of IFNγ by T cells.
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The role of dendritic cells in mycobacterium-induced granulomas
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(B) DCs found within chronic granulomas with low expression of T cell stimulatory molecules and secreting IL-10, will be liable to support an anergic or regulatory T cell response. This would result in less local IFNγ production, and thus, maintain mycobacterial latency inside macrophage.
Mycobacteria tuberculosis target DC-SIGN through ManLAM to suppress cellular immune responses mediated by dendritic cells!

M. tuberculosis infects macrophages through the mannose receptor and/or DCs through DC-SIGN. Macrophages/DCs that are infected with M. tuberculosis secrete the virulence factor ManLAM that binds to DC-SIGN on DCs that are attracted to the inflammatory site.

Recognition of M. Tuberculosis by Toll-like receptors (TLRs) expressed by DCs results in the activation of nuclear factor-kappaB (NF-kappaB), leading to the activation/maturation of DCs, as observed by increased expression of co-stimulatory molecules CD80, CD83 and CD86. DC maturation leads to the production of inflammatory cytokines and immune activation to enhance T-cell responses to eliminate the pathogen.

Increased secretion of ManLAM by infected macrophages or DCs targets DC-SIGN and results in inhibitory signals that interfere with the TLR-activating stimuli that lead to DC maturation. The ManLAM–DC-SIGN interaction results in inhibition of DC maturation and induction of the immunosuppressive cytokine interleukin-10 (IL-10), thereby preventing an efficient cellular immune response against M. tuberculosis infection.
Main features of tuberculosis: from infection to host defence or host immunosuppression!

Cytokine-mediated activation of macrophages is necessary for the containment of *M. tuberculosis* infection!

Different *MTB* cell wall components are recognized by different PRR!
I *Mycobacteria tuberculosis*, ATTIVI NEL GRANULOMA, POSsono SFUGGIRE DA ESSO......
Consequences of dendritic cell migration out of chronic granulomas: dissemination and T cell priming.

(A) DCs containing viable mycobacteria migrate out of chronic primary lesions. In cases of miliary TB, mycobacterial dissemination first reaches vascular organs liver, spleen, bone marrow, and brain. However, dissemination may ultimately reach all organs of the body.

(B) Dissemination of viable bacilli or mycobacterial antigen from the granuloma to the draining lymph nodes within DCs. During acute infection this process is mediated by chemokine receptor 7, and possibly 8 on DCs in response to ligands CCL19 and CCL21, and CCL1, respectively, expressed in the lymphatics and in the lymph nodes. Newly primed mycobacteria-specific T cells would migrate out of the lymph node to chronic lesions to maintain cellular immunity.
TUBERCOLOSI POLMONARE
The memory response to the challenge with *M. tuberculosis* antigens: a cutaneous type IV hypersensitivity (DTH) reaction, the “tuberculin type”
In Panel A, after vaccination, conventional BCG is taken up by the macrophage into a membrane-bound phagosome. BCG produces urease, which counters the normal acidification of the phagosome, establishing a neutral pH. Inside the neutral phagosome, BCG thrives and Antigens are processed through the MHC class II pathway for presentation to CD4+ T cells. Grode et al.1 recently tested a new recombinant BCG vaccine, in which the urease gene is deleted and a copy of the gene encoding lysin, taken from Listeria monocytogenes, is inserted (Panel B). The modified BCG vaccine is taken up into the phagosome where, in the absence of urease, normal acidification occurs. The recombinant BCG produces lysin, which punches holes in the surrounding membrane, allowing BCG to escape into the cytoplasm, which leads to apoptosis and killing of the bacillus. Antigens from the BCG that escape into the cytoplasm are processed through the MHC class I pathway and presented to CD8+ T cells. As a result, immunization with the improved BCG vaccine leads to a broader and more effective immune response and better protection against mycobacterial infection.
Un altro tipico granuloma immunologico è quello che si forma nella LEBBRA!

Leprosy, a chronic infectious disease caused by Mycobacterium leprae, affects primarily the skin, mucous membranes, and peripheral nerves. It is characterized by a spectrum of symptoms, largely a result of the immunological response of the host to the antigens of M. leprae. At one pole [lepromatous leprosy (LL)] there is an enormous growth of the bacilli in the tissues resulting from a specific and profound lack of cell-mediated immunity (CMI) of the host to M. leprae (Th2). At the opposite pole [tuberculoid leprosy (TT)], patients are responsive to M. leprae antigen, and there are few bacilli present in the lesions (Th1).

IL GRANULOMA delle LEBBRA o LEPROMA!

The disease clinically sometime is characterised by erythematous or hypopigmented cutaneous macules with peripheral sensory and motor neuropathies!

**Facial tuberculoid leprosy: case report**

British Journal of Oral and Maxillofacial Surgery, Volume 49, Issue 1, January 2011, Pages 70–72

K. Ali, G. Sittampalam, M.A. Malik
Primary syphilis usually begins with a single, painless, well-demarcated ulcer (chancre) with a clean base and indurated border. When the patient seeks treatment for signs or symptoms, the solitary chancre of primary infection may still be visible, or it may have progressed to secondary infection (e.g., rash, lymphadenopathy) or tertiary infection (e.g., gummatous lesions).

Primary syphilis!

Diagnosis and management of genital ulcers.
Roett MA, Mayor MT, Uduhiri KA.
T. pallidum strain types identified throughout the world!

The strain type information, years of collection, and the frequency of each strain type from each location!
The natural history of untreated syphilis in immunocompetent individuals!

Syphilis: using modern approaches to understand an old disease.

Ho EL, Lukehart SA.
Department of Neurology, University of Washington School of Medicine, Seattle, Washington, USA.
Histoplasmosis!

Every year, hundreds of thousands of people worldwide get a lung disease called histoplasmosis. It's transmitted through airborne spores that you breathe into your lungs when you work in or around soil that contains a fungus called *Histoplasma capsulatum*. Farmers, landscapers, construction workers and people who have contact with bird or bat droppings are especially at risk for histoplasmosis.
I MONOCITI/MACROFAGI ed I NEUTROFILI ATTIVATI DA STIMOLI IMMUNOLOGICI (CITOCHINE PRODOTTE DA TH1, TH2 ed anche TH17!) PARTECIPANO ANCHE ALLA PATOGENESI DI MALATTIE INFILAMMATORIE CRONICHE POUCO GRANULOMATOSE, MA SPESSO DIFFUSE e/o FIBROTICHE: L’ASMA CRONICA, LA DERMATITE ALLERGICA, LA PSORIASI e LE IBD*!

*IN ALCUNI CASI DI IBD, SI POSSONO TROVARE GRANULOMI!

| Crohn disease (inflammatory bowel disease) | Immune reaction against intestinal bacteria, possibly self antigens | Occasional noncaseating granulomas in the wall of the intestine, with dense chronic inflammatory infiltrate |
The special case of Asthma: airway inflammation and remodeling!

Barnes, Nature Reviews Drug Discovery, 2004
It has been clearly shown that asthma is caused by multiple environmental factors in combination with several major and minor susceptibility genes, which can give origin to many different forms or phenotypes of disease. These phenotypes include:

- allergic asthma, for sure the most common form of the disease;
- severe steroid-resistant asthma;
- asthma induced by air pollutants,
- obesity;
- aspirin;
- and exercise.
Role of Th2 lymphocytes in classical asthma pathogenesis!

Cytokines and chemokines produced by Th2 cells, including GM-colony stimulatory factors, IL-4, IL-5, IL-9, IL-13, and those produced by other cell types in response to Th2 cytokines (CCL11) account for most pathophysiologic aspects of allergic disorders, such as production of IgE antibodies; recruitment and activation of mast cells, basophils, and eosinophils granulocytes; mucus hypersecretion; subepithelial fibrosis; and tissue remodeling.
The classical Th2- and eosinophils-mediated Asthma!
CD4+ T effector lymphocytes are distinguished in different subsets on the basis of their patterns of cytokine secretion. Th1 cells, thanks to IFN-γ production, are responsible for cell-mediated immunity against intracellular pathogens. Th2 cells, through the production of IL-4, provide some degree of protection against helminthes, and Th17 cells, via IL-17, promote neutrophils recruitment for the clearance of bacteria and fungi. However, beyond their protective role, these T-helper subsets can also be involved in the pathogenesis of several inflammatory diseases. Asthma is an inflammatory disease characterized by different clinical phenotypes. Allergic asthma is the result of an inflammatory process driven by allergen-specific Th2 lymphocytes, whereas Th17 cells are mainly involved in those forms of asthma, where neutrophils more than eosinophils, contribute to the inflammation. The identification in allergic asthma of Th17/Th2 cells, able to produce both IL-4 and IL-17, is in keeping with the observation that different clinical phenotypes can coexist in the same patient. In conclusion, a picture in which different T-cell subpopulations are active in different phase of bronchial asthma is emerging, and the wide spectrum of clinical phenotypes is probably the expression of different cellular characters playing a role in lung inflammation.
Identification of Th17/Th2 lymphocytes and their possible role in asthma pathogenesis

Recently we described a novel subset of human circulating memory CD4 T cells that produce both IL-17A and IL-4 (83). This previously unknown population of Th17/Th2 lymphocytes was more represented in the circulation of patients with allergic asthma than in healthy donors, suggesting a possible role in the pathogenesis of the disease. The existence of human Th17 cells able to produce IL-4, IL-5, IL-9, and IL-13 in addition to IL-17A, IL-8, and IL-22, was initially shown on T-cell clones generated from circulating CD4+ CD161+ CCR6+ T cells and then confirmed on freshly derived circulating CD4 T cells of healthy donors and asthmatics. The proportion of Th17/Th2 cells was extremely low in healthy subjects, whereas their numbers appeared to be significantly higher in the circulation of patients with chronic severe asthma. The proportion of Th17/Th2 cells was found to be significantly higher in Der p 1-, than in polyclonal-expanded, peripheral blood mononuclear cells (PBMC), derived from asthmatic Der p 1 allergic donors, suggesting that Th17/Th2 cells present in the circulation of atopic patients with asthma are specific for the sensitizing allergen. The observation that Th17 cells expressed the IL-4R, and were susceptible to the activity of IL-4, which induced STAT6 phosphorylation, whereas Th2 cells did not express IL-1RI and IL-23R, and did not show STAT3 or STAT4 phosphorylation in response to IL-23, led us to hypothesize that an IL-4–rich microenvironment could favor the switch of allergen-specific Th17 lymphocytes toward the Th17/Th2 phenotype (83). Allergen-specific classic Th2 cells that respond to allergen in the lung could also modulate, thank to their ability to produce IL-4, Th17 lymphocytes specific for invading pathogens, toward the Th17/Th2 phenotype. This hypothesis is in keeping with our previous demonstration of the high plasticity of human Th17 cells (84). Another possibility is that allergen-specific Th2 lymphocytes become IL-17 producers in response to inflammatory stimuli. In fact it has been recently reported in mice that the proinflammatory cytokines IL-1β, IL-6, and IL-21 could directly induce the up-regulation of IRF4 and RORγt gene expression and the production of IL-17 in classical Th2 memory cells in vitro, suggesting a substantial phenotypic plasticity in response to inflammatory cues (85).

In any case, the demonstration of the existence in vitro and ex-vivo of CD4 cells able to produce both Th17-related and Th2-related cytokines, together with their increase in the circulation of patients with asthma, raises the important question of the pathophysiological role of this novel subset in allergic asthma. The presence of these cells at the level of bronchoalveolar lavage or bronchial biopsies of patients with asthma has never been reported in humans. However, the existence of the same subset of memory/effector T lymphocytes that coexpress the transcription factors GATA3 and RORγt and coproduce Th17 and Th2 cytokines has recently been shown.
In the acute phase of atopic dermatitis, Langerhans' cells are activated on binding of allergens by means of specific IgE and FcRI. They produce monocyte chemotactic protein 1 (MCP-1) and interleukin-16. Allergen-derived peptides are presented to T cells by Langerhans' cells that induce a Th2 profile. After migration into the skin, the recruited monocytes differentiate into inflammatory dendritic epidermal cells (IDEC) and produce the proinflammatory cytokines interleukin-1, interleukin-6, and tumor necrosis factor (TNF-). Their secretion of interleukin-12 and interleukin-18 contributes to the switch from Th2 to Th1/0 and thereby leads to the chronic phase of the disease.

Causes and potential cytokine networks in psoriatic lesions

- Initiating events:
  - ENVIRONMENT: HSPs, TLR agonist, Cytokines, Infection
  - T cell: Tissue-resident
  - CD11c+ DC (TIP-DC)

- Amplification of inflammation:
  - T helper 17 (Th17)
  - IL-17
  - IL-12, IL-23
  - IFN-γ, TNF, LT
  - IL-17
  - IL-22
  - IL-1
  - IL-6
  - IL-20

- End response:
  - Vascular response
  - Fibroplasia
  - KGF
  - ECGF
  - VEGF
  - PDGF
  - TGF-β
  - STAT1-P
  - STAT3-P
  - NF-κB
  - IFN-response genes
  - Genes with composite IFN and NF-κB response elements
  - NF-κB-regulated genes

LA PSORIASI!
PATOGENESI DELLA PSORIASI!

UNO DEI PRINCIPALI EVENTI SCATENANTI DELLA PSORIASI È RAPPRESENTATO DAL PEPTIDE ANTIMICROBIOTICO CATELICIDINA LL-37.

CHE SI LEGA AL DNA SELF E DIVENTA UN AUTOANTIGENE.
ESSO, CAPTATO DALLE CELLULE DENDRITICHE CUTANEE VIENE A STIMOLARE LINFOCITI AUTOREATTIVI TH1 E TH17 CHE PRODUCONO CITOCHINE CHE ATTIVANO CHERATINOCITI, MONOCITI E NEUTROFILI, PRODUCENDO LE LESIONI INFIAMMATATORIE CUTANEE TIPICHE DELLA PSORIASI!
Proposed scheme of the evolution of a psoriatic lesion from initiation to maintenance of disease!!!
Interleukin-23 and interleukin-17: Importance in pathogenesis and therapy of psoriasis.

Mudigonda P, Mudigonda T, Feneran AN, Alamdari HS, Sandoval L, Feldman SR.
Department of Dermatology, Center for Dermatology Research, Wake Forest School of Medicine, Winston-Salem, North Carolina.

Emerging data in mice and humans reveals a critical contribution of Th17-associated cytokines, particularly interleukin-(IL)-23 and IL-17, in the pathogenesis of psoriasis. The IL-23/Th17 pathway is a therapeutic target for biologic agents and systemic therapies in psoriasis treatment. A literature search was performed to review and summarize the current evidence on IL-17 and IL-23 as a basis for understanding the use of anti-IL-17 and anti-IL-23 agents for psoriasis therapy. Using PubMed, recent articles were identified pertaining to IL-17, IL-23, and psoriasis. Signaling via the heterodimeric IL-23 receptor induces production of IL-17, which stimulates production of proinflammatory keratinocyte cytokines that mediate the psoriatic response. An overexpression of IL-23, IL-17, or Th17 cells in transgenic mice is associated with the development of inflammatory disease. Both IL-17 knockout mice and humans with a genetic IL-17 deficiency are susceptible to extracellular and intracellular pathogens. This suggests a potential for adverse effects from clinical administration of anti IL-23-p40/IL-17 therapies. Anti-p40 antibodies, briakinumab and ustekinumab, were tolerated in clinical trials and substantially improved psoriasis. Further trials of anti IL-17 therapies are needed to assess their clinical use and potential for infection and other adverse events.

One novel agent that directly targets IL-17 is currently undergoing clinical trial. Secukinumab (AIN57), a monoclonal antibody that neutralizes IL-17A, was tested on patients with plaque-type psoriasis (n=36) in a randomized, proof-of-concept clinical trial. Dermal IL-17A+ CD3+ T cells and mRNA expression of IL-17 and IL-22 were markedly reduced.

Additional biologic agents, such as LY-24398121, an anti-IL-17 monoclonal antibody, and AMG-827, an anti-IL-17R agent, are also currently being evaluated in phase II clinical trials.

Efforts to target a different downstream effector are seen in a phase I clinical trial being completed for an IL-22 antibody, fezakinumab.

NUOVE TERAPIE DELLA PSORIASI!
Secukinumab prevents IL-17A binding to its receptor, inhibiting production of pro-inflammatory mediators!

Secukinumab: A New Treatment Option for Psoriatic Arthritis.
LE IBD (INFLAMMATORY BOWEL DISEASES) SONO:

- Crohn’s disease (CD)
- Ulcerative colitis (UC)
Mucosal inflammation is regulated by the interplay of resident microbiota, intestinal epithelium, and the mucosal immune system!

**Table 3  Candidate etiologies for inflammatory bowel disease**

<table>
<thead>
<tr>
<th>Impairment of mucosal barrier function</th>
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<tr>
<td>Defective innate immune control mucosal microorganisms</td>
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<tr>
<td>Mucosal dysbiosis or specific microbial pathogens</td>
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<td>Defective mucosal immunoregulation</td>
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Increased epithelial permeability may be important in the development of chronic gut T cell–mediated inflammation. CD4 T cells activated by gut antigens in Peyer's patches migrate to the LP. In healthy individuals, these cells die by apoptosis. Increased epithelial permeability may allow sufficient antigen to enter the LP to trigger T cell activation, breaking tolerance mediated by immunosuppressive cytokines and perhaps T regulatory cells. Pro-inflammatory cytokines then further increase epithelial permeability, setting up a vicious cycle of chronic inflammation.
THE MECHANISMS OF MICROBIOTA INHERITANCE!!!

- Care giving
  - Oral transfer from kissing
  - Topical from skin with touching

- Breast Feeding
  - Through breast milk, topical from skin

- Direct Intrauterine Seeding
  - Carriage into womb via dendritic cells
  - Access to womb through vagina
  - Access through bloodstream

- Passage Through Birth Canal or C-Section
  - Pregnancy shifts vaginal biome
  - Smearing with bacteria during vaginal delivery
  - Transference of skin bacteria during C-section

Host-microbiota neuroendocrine interactions influencing brain and behavior

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Keywords: hormones, neuroendocrine, enteric nervous system, signaling, behavior, microbial endocrinology
Food ingested by the host contains both the substrates needed for neurochemical production by the host and the microbiota as well as fully functional neuroactive components (1).

The microbiota in the gut is capable of either forming neurochemicals from the substrates present in the ingested food; or responding to the neuroactive food components themselves; or responding to neurochemicals secreted into the gut by components of the host enteric nervous system (2).

Neurochemicals produced by the microbiota in the gut have two pathways by which to influence the host; they can either be taken up from the gut into the portal circulation (3) or they can directly interact with receptors found on components of the enteric nervous system which innervates the complete length of the gastrointestinal tract (2).

Once in the portal circulation, microbiota-derived neurochemicals can influence components of the nervous system and ultimately the brain (4). Microbiota-derived neurochemicals can also influence components of the nervous system such as the brain through enteric nervous system-central nervous system communication (5). The result of either pathway (4) or (5) on the brain may result in an alteration of behavior or cognition (6) as well as food preferences and appetite (7).

As described in the text, this should not be viewed as a one-way direction of only gut-to-brain since the brain may influence the composition of the microbiota through the specific release of neurochemicals into the gut lumen (2).

The study of the production and recognition of neurochemicals that are exactly the same in structure to those produced in the vertebrate organisms is known as **microbial endocrinology**.
The microbial endocrinology-based pathways by which neuroactive compounds produced by both the host and the microbiota can serve as a mechanism by which the brain and behavior can be modulated within the microbiota-gut-brain axis.
Many factors contribute to the tolerance of commensal flora!!!

**commensal bacteria:**
- deficient escape mechanisms against mucus trapping
- deficient traits for epithelial adherence and invasion
- low endotoxicity (non-stimulatory LPS)
- anti-inflammatory products

**mucosal epithelium:**
- tight junctions (regulated transfer of commensal antigens)
- defective sensing of PAMPs
- rapid sensing for invasive microorganisms
- strong antimicrobial crypt functions (defensins)

- Lamina propria contains tolerogenic DC, macrophages, and regulatory T cells producing anti-inflammatory cytokines (IL-10 and TGF β) in response to commensal bacteria
- DC-derived retinoic acid (RA) and TGF β promote the differentiation and the activation of T reg cells in the gut
Treg cells inhibit inflammatory bowel diseases!

1. Inflammatory bowel disease and colitis result from autoreactive T cells in the lamina propria.
2. The disease can be treated by transfer of CD4 CD25 T\textsubscript{reg} cells, which home to mesenteric lymph nodes and the colon.
3. CD4 CD25 T\textsubscript{reg} cells proliferate and inhibit the pathogenic effector T cells.
4. After inflammation resolves, CD4 CD25 T\textsubscript{reg} cells remain in clusters with dendritic cell and pathogenic effector T cells.
Bacterial metabolites fight intestinal inflammation through Treg!

Commensal bacteria metabolize fiber and generate short-chain fatty acids. These fatty acids are ligands for GPR43 expressed by Treg cells and stimulate their expansion and immune-suppressive properties such as the production of IL-10, thereby controlling proinflammatory responses in the gut.

Bollrath J1, Powrie F.
Bacterial metabolites fight intestinal inflammation through Treg!

I batteri commensali metabolizzano inoltre fibre e generano nell'intestino acidi grassi a catena corta (SCFA) (circa 40 mM di acetato e 20 mM di propionato e butirrato). I principali effetti immunomodulatori delle fibre alimentari e dei SCFA sono: l'esclusione competitiva dei batteri, per cui una dieta ricca di fibre favorisce i batteri commensali e limita l'accesso dei patogeni; aumento della secrezione di muco da parte delle cellule epiteliali intestinali e di IgA; potenziamento dei meccanismi di riparo e dell'integrità epiteliale; sviluppo di linfociti Treg, che regolano la tolleranza immunologica ed un ambiente intestinale anti-inflammatorio. Gli SCFA (principalmente l'acetato) sono anche trasportati dall'intestino al sangue e possono così modulare le cellule di midollo osseo e di altri tessuti, e sono in grado di attraversare la placenta o essere secreti nel latte materno.

Immunology. Feed your Tregs more fiber.
Bollrath J1, Powrie F.
IL CONTROLLO DEI LINFOCITI T DELLA RISPOSTA IMMUNITARIA È OPERATO ANCHE DALLA VITAMINA A E DA METABOLITI INTESTINALI PRODOTTI DAL CIBO CHE INGERIAMO!
Attraverso l'acido retinoico (RA), il metabolita derivato dalla trasformazione del retinolo, la vitamina A controlla numerose risposte immunitarie.

In condizioni fisiologiche, l’RA mantiene la tolleranza orale e l'integrità della barriera mucosale grazie alla capacità di indurre Treg e Th17.

Durante una risposta infiammatoria o immunitaria, le DC, in presenza di RA, inducono l’attivazione e la polarizzazione dei linfociti T CD4+ nonché il loro corretto reclutamento a livello mucosale tramite l’induzione dell’espressione del recettore chemochininico CCR9 e dell’integrina α4β7 e l’inibizione del recettore CLA che controlla l’homing linfocitario cutaneo. Le DC influenzano inoltre, mediante l’RA e le citochine IL-5 e IL-6, la localizzazione mucosale delle plasmacellulare producenti IgA.
Composti come l’indolo-3-carbinolo derivati da broccoli, cavolfiori e cavolo sono convertiti dall’esposizione agli acidi gastrici in metaboliti, quali l’indolo [3,2-b] carbazolo, che legano ad alta affinità il recettore AhR (aryl hydrocarbon receptor). Questi eterodimerizzano con la proteina Arnt e traslocano nel nucleo dove regolano la trascrizione di geni coinvolti nel controllo della sopravvivenza di cellule linfoidi innate, nello sviluppo di Th17 e nella produzione di IL-22 e di fattori anti-microbicidi, responsabili del mantenimento dell’integrità della barriera epiteliale e dell'immunità ed omeostasi intestinale. AhR è anche attivato dalla chinurenina, generata per azione dell’indoleammina 2,3-diossigenasi dal triptofano, presente in carni rosse, pesce, uova, e verdure, e dall’indolo-3-aldeide, derivato del triptofano prodotto dai lattobacilli intestinali!
Dietary derived influences on intestinal immunity!

Fattori dietetici e nutrienti influenzano potentemente l'immunità intestinale. La vitamina A può agire sia sulle cellule T o le cellule dendritiche per promuovere le risposte immunitarie mucosali. La vitamina D ha dimostrato promuovere lo sviluppo dei linfociti intraepiteliali (IEL) e la secrézione macrofagica di peptidi microbicidi. I ligandi degli AHR regolano i linfociti intraepiteliali (IEL). Le fibre contenute nella dieta favoriscono da parte dei batteri commensali il metabolismo degli acidi grassi a catena corta (SCFA) che limitano la funzione dei neutrofili e rafforzano le barriere epiteliali!

Interdependence of diet, immune and commensal interactions: evidence now exists for bidirectional communication between the three key factors in the GI tract: diet, immunity and commensal microflora.

Dietary and commensal derived nutrients: shaping mucosal and systemic immunity.
Spencer SP1, Belkaid Y.
Proposed causes of dysbiosis of the microbiota

The composition of the microbiota can shape a healthy immune response or predispose to disease!
Possible mechanisms leading to IBD!!

**activation** \(\uparrow\) **proliferation/survival** \(\uparrow\) **apoptosis** \(\downarrow\)

- **physiological inflammation**
- **pathological inflammation** (IBD)
- **controlled inflammation**
Inflammatory Bowel Disease (IBD): a dysregulated immune response against commensal bacteria, in genetically predisposed individuals

**Crohn’s disease (CD)**
- Th1, Th17
- IFN\(\gamma\), TNF, IL-12, IL-23, IL-18, IL-15, chemokines

**ulcerative colitis (UC)**
- Th2-like
- IL-13, IL-4, IL-5, chemokines

Genes

Replicated Crohn’s disease loci
- NOD2
- IBD5
- IL23R
- ATG16L1
- Chr 5p13.1
- Chr 5q33.1 (IRGM)
- Chr 10q21.1

Additional replication required
- NCF4
- PHOX2B
- PTPN2
- TNFSF15
- Chr 16q24.1 (FAM92B)
New not invasive tools that can be used as a marker of localized inflammation:

the phagocyte-specific S100 calcium-binding proteins!
The phagocyte-specific S100 calcium-binding proteins!

Phagocyte-specific calcium-binding S100 proteins as clinical laboratory markers of inflammation.
Foell D, Frosch M, Sorg C, Roth J.

• The first member of the S100 family of proteins, which are part of the larger group of calcium-binding proteins, was isolated in 1965.

• The S100 proteins comprise the group of calgranulins, are pro-inflammatory molecules expressed and secreted by phagocytes. The three members of this group, S100A8, S100A9 and S100A12 are over-expressed at the site of inflammation.

• The heterodimer of S100A8 and S100A9, known as "leukocyte protein L1", is now still called calprotectin by some research groups.

• Both the complex S100A8/S100A9 that S100A12 are useful diagnostic factors of inflammation especially in the case of arthritis, chronic inflammation of the lungs and intestinal diseases.

• They are index of activation of phagocytes more than any other parameter of inflammation. These proteins are able to detect minimal residual levels of inflammation and can be predictive for the prognosis of the patient.
Fecal calprotectin (FC) has been proposed as a useful and non-invasive marker of acute intestinal inflammation.

We summarize recent evidences on FC, providing practical perspectives on its diagnostic and prognostic role in different gastrointestinal conditions.

RESULTS:
Most of relevant data derived from studies on inflammatory bowel disease (IBD). FC concentrations (FCCs) showed a good diagnostic precision for separating organic and functional intestinal diseases and well correlated with IBD activity. FCCs were higher in subjects with NSAID enteropathy, but the actual correlation between FC and endoscopy is under investigation.

CONCLUSIONS:
FC has been widely proposed as a filter to avoid unnecessary endoscopies. Nevertheless, it should not be considered as a marker of organic intestinal disease at all; rather it represents a marker of "neutrophilic intestinal inflammation". In IBD, more and larger studies are needed to confirm FC's capacity to correlate with IBD extent, to predict response to therapy and relapse, and the presence of a subclinical intestinal inflammation in asymptomatic first-degree relatives of patients.
**Calprotectina**

**Indice di infezione intestinale**

Calprotectina è il test per individuare pazienti con possibile infezione dell'intestino: scopri i test disponibili e il loro funzionamento.

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**Calprotectina: diagnosi e test**

**Utilizzo in età pediatrica**

**Utilizzo in età adulta**

Calprotectina nelle feci

**Device per prelievo feci**

CalFast

Calprest

**BIBLIOGRAFIA**

**FAQ**

**IRRITABILE (IBS)**

**SINDROME DELL’INTESTINO CRONICHE INTESTINALI (IBD)**

**MALATTIE INFiammatorIE ORGANICHE**

**MORBODI CRONICI**

**RAGIONI DELL’INTESTINO**

**Ciste**

**Malattie Infiammatorie Croniche Intestinali**

**COLITE ULCEROSA**

**UO. IMMUNOLOGIA- IMMUNOPATOLOGIA DL 385**

Responsabile P.F. Prof. Fabrizio Mainiero

Tel: 06 49970966

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**Calprotectina** è il test immunoenzimatico di Eurospital che consente di verificare, in modo accurato e non invasivo, la presenza di uno stato infiammatorio a carico del tratto intestinale.

Calprotectina viene utilizzata per escludere una diagnosi differenziale fra patologie di tipo organico (Malattie Infiammatorie Croniche Intestinali - MICI, Note anche come Inflammatory Bowel Disease - IBD) e di tipo funzionale (Sindrome dell'Intestino Irritabile - SII, Irritable Bowel Syndrome - IBS). Se Calprotectina fornisce un risultato negativo, si può, con quasi assoluta certezza, escludere un’infezione a carico della mucosa intestinale.

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**UN TEST SEMPLICE E ACCURATO**

Fino ad oggi, per valutare lo stato infiammatorio della mucosa intestinale era necessario ricorrere ad esami invasivi (colonoscopia e conseguente esame istologico). Di recente, però, ha trovato sempre più credito l’uso di marcatori non invasivi: tra questi, uno dei più attendibili e sicuri è il dosaggio quantitativo di calprotectina nelle feci.

Il principio diagnostico di Calprotectina si basa sulla determinazione quantitativa della calprotectina, una proteina antimicrobica presente nei neutriffili che, in presenza di processi infiammatori a carico dell’intestino, viene rilasciata nel lume intestinale e pertanto può essere rilevata nelle feci.

Il dosaggio quantitativo di calprotectina nelle feci è una importante strumento diagnostico per il sopralluogo del tratto intestinale.

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**SENSIBILITÀ E SPECIFICITÀ**

La determinazione della calprotectina fecale viene impiegata per la diagnosi differenziale fra IBD ed IBS. I campioni con una concentrazione di calprotectina superiore a 50 mg calprotectina/kg devono essere considerati positivi ai test. Nei soggetti adulti, il valore medio della calprotectina è di 25 mg calprotectina/kg.
Progressive multifocal leukoencephalopathy (PML) is caused by reactivation of latent JC virus, leading to the death of myelin-producing oligodendrocytes. Once seen primarily in severely immunosuppressed states, PML has now emerged as a rare adverse effect in natalizumab (anti-α4 integrin)-treated MS and IBD patients.

Anti-α4 integrin mAb interferes with pathogenic T cell migration to CNS (multiple sclerosis) and to inflamed gut (IBD).
PML: Progressive multifocal leukoencephalopathy (PML) is caused by reactivation of latent JC virus, leading to the death of myelin-producing oligodendrocytes. Once seen primarily in severely immunosuppressed states, PML has now emerged as a rare adverse effect in natalizumab (anti-α4 integrin)-treated MS and IBD patients.
Faecal microbiota transplantation (FMT) has undergone dramatic progression over the past year and continues to evolve as knowledge of the gastrointestinal microbiota (GiMb) develops!

**KEY POINTS**

- The gastrointestinal microbiota, its active role in health and disease and the therapeutic potential of FMT are areas of great global interest undergoing rapid developments and advances.

- The efficacy of FMT in multiple relapsing CDI and its superiority over antibiotic therapy is now unequivocal.

- FMT is an exciting potential therapy for IBD with encouraging case reports and series especially in ulcerative colitis, though at present controlled trial data are lacking.

- FMT may have therapeutic applications for a wide range of not only gastrointestinal but also systemic conditions (e.g. diabetes, obesity, autoimmune phenomena) found to have their pathogenesis related to gastrointestinal dysbiosis.

- There are several products under development, and in the future, FMT will likely shift from the use of crude, fresh whole stool to highly processed, filtered, frozen and potentially cultured formulations.

_Borody TJ, Brandt LJ, Paramsothy S._
A 46-year-old woman with a 2-year history of Crohn's colitis was treated with a single, large volume nasojejunal* infusion of FMT over 6h for concurrent CDI.

(a, b) Descending colon and caecum (respectively), pre-FMT.

(c, d) Descending colon and caecum (respectively), 12 years post-FMT.

Structure completely normalized!

*A nasojejunal or NJ-tube is passed through the nares (nostril), down the esophagus, and through the stomach into the jejunum, the middle section of the small intestine. In some cases, a nasoduodenal or ND-tube may be placed into the duodenum, the first part of the small intestine!
A 38-year-old man with a 6-year history of ulcerative colitis, concurrent multiple sclerosis, sacroileitis and sclerosing cholangitis was treated with an initial transcolonoscopic FMT infusion, followed by over 100 FMT enemas during the next 12 months. After 4 weeks of daily FMT enemas, the patient's IBD symptoms had dramatically improved, liver biochemical tests had normalized and sacroileitis pain was absent.

(a, b) Transverse colon and hepatic flexure (respectively), pre-FMT.

(c, d) Transverse colon and hepatic flexure (respectively), post-FMT without bowel prep.

(e) Liver biochemical tests immediately prior to FMT, and 12 months post-FMT.
Clinical and experimental studies reveal that the colonic microbiota can enhance or ameliorate intestinal and systemic inflammatory diseases. Because of its potential to enhance resistance to infection and to reduce inflammatory diseases, targeted manipulation of microbial populations is a growing focus of investigation. The most dramatic manipulation of the intestinal microbiota involves fecal microbiota transplantation (FMT) from healthy donors to individuals with specific diseases. Remarkable clinical effectiveness of FMT has been demonstrated for IBD and recurrent Clostridium difficile infection and ongoing studies are investigating FMT for other diseases. Transplantation of complex microbial populations to recipients likely triggers mucosal immune responses that, depending on the microbiota composition and the recipient's genotype, could range from pro- to anti-inflammatory. The impact of FMT on the recipient immune system is complex and unpredictable. Ongoing discovery of commensal microbes and investigations of their impact on the host will lead to the development of new probiotic agents and microbial consortia that will eventually replace FMT.
IL TRAPIANTO DEL FECALOMA (FMT) AL POLICLINICO UMBERTO I°!

Direzione Sanitaria

Azienda Policlinico Umberto I — Roma
Protocollo Generale
Partenza
PROT. n. 0002906 del 29/01/2015

Ai Direttori DAI
Ai Direttori UOC
e per loro tramite
e p.c.
Al Prof. Vincenzo Vullo
   Direttore DAI Malattie Infettive
Al Prof. Guido Antonelli
   Direttore DAI Medicina Diagnostica
Al Prof. Enrico Corazzari
   Direttore UOC Gastroenterologia A
Al Prof. Carlo Mancini
   Direttore UOC Microbiologia
Alla Prof.ssa Gabriella Cancrini
   Resp. UOF Parasitologia
Alla Prof.ssa Anna Teresa Palamara
   Coordinatore Sezione di Microbiologia,
   Dipartimento di Sanità Pubblica e
   Malattie Infettive
   Università Sapienza di Roma
Al Dott. Giancarlo Ferretti
   Referente Infettivologo al Progetto

Oggetto: attivazione della procedura inerente il trapianto di microflora fecale per il trattamento della colite da Clostridium difficile

Con la presente si intende dare attuazione alla procedura condizionata dal Protocollo per il trapianto di microflora intestinale approvato dal Comitato Etico con riferimento n. 3336, in data 24 luglio 2014 (che si allega).
Il presente protocollo si applica al trattamento dei pazienti, degenti in questo nosocomio, affetti da Clostridium difficile (Cd), con recidiva di infezione e/o con episodio di malattia severa, rappresentando una terapia innovativa nell’ambito della cura della colite da Cd.

ALLEGATO N. 1
PROTOCOLLO “TRAPIANTO FECALE DI MICROBIOTA”

Proposta di trattamento delle infezioni ricorrenti e severe da Clostridium difficile con trapianto fecale di microflora e contestuale analisi della composizione e delle variazioni del microflora intestinale nel donatore e nel ricevente pre- e post-trapianto, individuando eventuali specie batteriche correlate all’infezione o curative della stessa.

Legenda terminologia

CD: Clostridium difficile
CDI: infezione da Clostridium difficile
FMT: trapianto fecale di microflora

Infezione ricorrente: infezione da Clostridium difficile che si ripresenta entro 8 settimane dopo un primo episodio di CDI, a condizione che i sintomi della precedente infezione siano completamente risolti dopo la conclusione del trattamento iniziale.

Infezione moderata: si intende una diarrea da CD associata ad altri segni o sintomi che non sono inclusi nei criteri della forma severa o complicata

Infezione severa: infezione da CD con valori plasmatici di albumina inferiori a 3 g/dl più uno dei seguenti criteri:
   - leucocitosi WBC > 15.000 cells/mm³
   - distensione addominale

Infezione complicata: uno dei seguenti criteri attribuibili alla CDI
   - presenza di megacolon tossico
   - ricovero presso un’unità di terapia intensiva
   - febbre > 38 C
   - livelli dei lattati sierici > a 2,2 mmol/L
   - alterazione dello stato di coscienza
   - leucocitosi WBC > 30.000 cells/mm³ o < 2000 cells/mm³
I MECCANISMI MOLECOLARI
DEL RIPARO
I meccanismi coinvolti nel riparo dei tessuti dipendono dall’intensità del danno e dal tipo di cellule coinvolte!!!

**Figura 14.3 - Rapporti esistenti tra tipo cellulare danneggiato, rigenerazione e “restituito ad integrum”**
The mechanisms underlying this response are not understood, but likely involve local production of growth factors and interactions of cells with the ECM. The extraordinary capacity of the liver to regenerate has made it a valuable model for studying this process, as restoration of normal tissue structure can occur only if the residual tissue is structurally intact, as after partial surgical resection. By contrast, if the entire tissue is damaged by infection or inflammation, regeneration is incomplete and is accompanied by scarring. For example, a liver abscess, which occurs in a liver abscess, leads to scar formation even though the remaining liver cells have the potential to undergo proliferation. The process involves the activation of different signaling pathways, including the growth factor family, which includes transforming growth factor alpha (TGF-α), hepatocyte growth factor (HGF), and epidermal growth factor (EGF). These factors act on their respective receptors, EGFR and MET, to initiate the transition from the G0 to G1 phase of the cell cycle, leading to cell proliferation and repair.

I MECCANISMI MOLECOLARI DELLA RIGENERAZIONE EPATICA!
DELLA RIGENERAZIONE ALLA REINTEGRAZIONE CONNETTIVALE (FIBROSI)!

Figura 3-31  Mechanisms of fibrosis. Persistent tissue injury leads to chronic tissue injury, loss of epithelial integrity.

- Limited injury
- Repeated or severe injury

Epithelium

Tissue injury, loss of epithelial integrity

Macrophages

T lymphocytes and other lymphoid cells

Cytokines (e.g., IL-13)

TGFβ

MMPs

Fibroblast recruitment and differentiation

Myofibroblasts

Extracellular matrix

FIBROSIS
La cinetica delle tre fasi del processo di riparazione
DALL’INFIAMMAZIONE AL RIPARO!
IL PROCESSO DI RIPARAZIONE DELLE FERITE!
IL PROCESSO DI RIPARAZIONE DELLE FERITE!

NORMAL

Infection or injury

TISSUE INJURY

Area of injury

INFLAMMATION

FORMATION OF GRANULATION TISSUE

SCAR FORMATION
IL PROCESSO DI RIPARAZIONE DELLE FERITE!
Le FERITE guariscono per I° o II° INTENZIONE!
RIPARAZIONE delle FERITE per I° e II° INTENZIONE!

HEALING BY FIRST INTENTION
- Scab
- Neutrophils
- Clot
- Mitoses
- Granulation tissue
- Macrophage
- Fibroblast
- New capillary

HEALING BY SECOND INTENTION
- Wound
- Contraction

24 hours
- Neutrophils
- Clot

3 to 7 days
- Mitoses
- Granulation tissue
- Macrophage
- Fibroblast
- New capillary

Weeks
- Fibrous union

Wound contraction
La riparazione: dai primi giorni

Tessuto di granulazione
...........a uno-due mesi!!!
Guarigione di una ferita cutanea per seconda intenzione!!!
LA CINETICA DEGLI EVENTI CHE CARATTERIZZANO IL PROCESSO RIPARATIVO DI UNA FERITA!
I MECCANISMI MOLECOLARI COINVOLTI NEL RIPARO SONO MOLTO COMPLESSI E COINVOLGONO:

- cellule;
- mediatori chimici;
- citochine e chemochine e fattori di crescita;
- componenti della matrice extracellulare (ECM), (glicosamminoglicani (GAG), quali l’eparina e l’acido ialuronico, proteine fibrose strutturali, quali collagene, vitronectina, laminine e fibronectina, proteine matricellulari, quali la SPARC/osteonectina, le trombospondine e la tenascina);
- enzimi proteolitici, quali il sistema attivatore del plasminogeno di tipo urochinas (uPA) e di tipo tissutale (tPA), e le metalloproteasi;
- molecole di adesione, quali integrine e caderine.
GLI EVENTI DEL RIPARO E I FATTORI E LE CELLULE CHE LI CONTROLLANO

<table>
<thead>
<tr>
<th>Fase</th>
<th>Fattori</th>
<th>Fonte</th>
<th>Effetti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulazione</td>
<td>XIIIa, TGF-α, TGF-β, PDGF, ECGF</td>
<td>Plasma, Piastrina</td>
<td>Coagulo</td>
</tr>
<tr>
<td>Infiammazione</td>
<td>TGF-β</td>
<td>Neutrofili</td>
<td>Attrazione di monociti/macrofagi e fibroblasti, differenziazione dei fibroblasti</td>
</tr>
<tr>
<td>Formazione del tessuto di granulazione</td>
<td>FGF basico, TGF-β</td>
<td>Monociti/macrofagi, poi fibroblasti</td>
<td>Legame di vari fattori al proteoglicano della matrice</td>
</tr>
<tr>
<td>Angiogenesi</td>
<td>VEGF</td>
<td>Monociti/macrofagi</td>
<td>Sviluppo dei vasi sanguigni</td>
</tr>
<tr>
<td>Contrazione</td>
<td>TGF-β1, β2</td>
<td>Varie</td>
<td>Comparsa di miofibroblasti, legati l’uno all’altro e al collagene, che si contraggono</td>
</tr>
<tr>
<td>Maturazione, arresto della proliferazione</td>
<td>TGF-β1</td>
<td>Piastrina, mononuclei/macrofagi</td>
<td>Accumulo di matrice extracellulare</td>
</tr>
<tr>
<td></td>
<td>Proteoglicano eparansolfato, decorina, interferone</td>
<td>Fibroblasti secretori</td>
<td>Captazione di TGF-β e di FGF basico</td>
</tr>
<tr>
<td>Rimodellamento</td>
<td>Aumento locale di ossigeno</td>
<td>Processo di riparazione</td>
<td>Soppressione della proliferazione dei fibroblasti e accumulo di collagene</td>
</tr>
<tr>
<td></td>
<td>PDGF-FGF</td>
<td>Piastrina, fibroblasti</td>
<td>Soppressione del rilascio di citochine</td>
</tr>
<tr>
<td></td>
<td>Metalloproteinasi della matrice, t-PA, u-PA, inhibitori tessutali delle metalloproteinasi</td>
<td>Formazione di capillari, cellulari epiteliiali</td>
<td>Induzione di MMP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Locale, non meglio specificata</td>
<td>Rimodellamento tramite crescita di nuovi vasi e ricostruzione della ECM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bilanciamento degli effetti delle MMP nel sito di riparazione in evoluzione</td>
</tr>
</tbody>
</table>
GLI EVENTI DEL RIPARO E I FATTORI E LE CELLULE CHE LI CONTROLLANO

<table>
<thead>
<tr>
<th>Fase</th>
<th>Fattori</th>
<th>Fonte</th>
<th>Effetti</th>
</tr>
</thead>
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<tr>
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<td>TGF-β1, Proteoglicano eparansolfato, decorina, Interferone</td>
<td>Piastrina, monociti/macrophagi, Fibroblasti secretori, Monociti plasmatici</td>
<td>Accumulo di matrice extracellular, Captazione di TGF-β e di FGF basico, Soppressione della proliferazione dei fibroblasti e accumulo di collagene, Soppressione del rilascio di citochine, Induzione di MMP</td>
</tr>
<tr>
<td>Rimodellamento</td>
<td>Aumento locale di ossigeno, PDGF-FGF, Metalloproteinasi della matrice, t-PA, u-PA, Inibitori tessutali delle metalloproteinasi</td>
<td>Processo di riparazione, Piastrina, fibroblasti, Formazione di capillari, cellulari epiteliari, Locale, non meglio specificata</td>
<td>Rimodellamento tramite crescita di nuovi vasi e ricostruzione della ECM, Bilanciamento degli effetti delle MMP nel sito di riparazione in evoluzione</td>
</tr>
<tr>
<td>Classe di Macromolecole</td>
<td>Componente alterato</td>
<td>Malattie</td>
<td>Organi affetti</td>
</tr>
<tr>
<td>--------------------------</td>
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</tr>
</tbody>
</table>
| **GLICOSAMMINOGLICANI (GAG)** | proteoglicani (da alterazioni del DTDST) | Displasia Diastrofica  
Displasia Epifissaria Multipla tipo 4  
Acondrogenesi tipo IB  
Atelosteogenesi tipo II | Cuta  
Articolazioni Ossa |
| | perlecan o | Displasia di Silverman-Handmaker | Cartilagine  
Scheletro |
| | chératansolfato, dermatansolfato, eparansolfato (accumulo da alterazioni di enzimi lisosomiali) | Mucopolisaccaridosi (MPS):  
I Sindrome di Hurler-Scheie  
II Sindrome di Hunter  
III Sindrome di Sanfilippo  
IV Sindrome di Morquio  
VI Sindrome di Maroteaux-Lamy  
VII Sindrome di Sly | Tutti gli organi |
| **COLLAGENI** | collagene I | Osteogenesi Imperfetta (OI)  
OI tipo Vrolik | Cartilagine  
Scheletro |
| | collagene II | Acondrogenesi tipo II (Langer-Saldu no) | Ossa |
| | collagene III e V | Sindrome di Ehlers-Danlos (EDS) | Articolazioni Vasi |
| | collagene IV | Sindrome di Alport | Rene |
| | collagene VI | Miopatia di Bethlem  
Distrofia Muscolare Congenita di Ullrich | Muscoli |
| | collagene X | Condrodisplasia Metafisaria tipo Schmid | Ossa |
| | collagene IX | Displasia Epifissaria Multipla tipo II | Ossa |
| | collagene VII | Epidermolisi Bollosa Distrofica (EBD) | Cuta  
Mucose |
| | collagene XVII | Epidermolisi Bollosa Giunzionale (EBG) non Herlitz | Cuta  
Mucose |
| **FIBRE ELASTICHE** | fibrillina I | Sindrome di Marfan | Articolazioni Vasi |
| | fibrillina II | Sindrome di Beals-Hecht | Articolazioni |
| **FIBRONECTINA** | | Assenza incompatibile con la vita | |
| **LAMININE** | laminina 2 | Distrofia Muscolare Congenita | Muscoli |
| | laminina 5 | Epidermolisi Bollosa Giunzionale (EBG) di Herlitz | Cuta  
Mucose |
| **TROMBOSPONDINE** | trombospodina 5 (COMP) | Pseudoacondroplasia  
Displasia Epifissaria Multipla tipo I | Articolazioni  
Ossa |
| **TENASCINE** | tenascina-X | Sindrome di Ehlers-Danlos | Articolazioni |
| **MATRILINE** | matrilina 3 | Condrodisplasia | Articolazioni Ossa |
| **FIBULINE** | fibulina 5 | Cutis Laxa (Cuta Lassa) | Cuta  
Vasi |
| | fibulina 3 | Malattia Leventinese  
Distrofia Retinica di Doyne | Occhi |
| | fibulina 1 | Sindrome delle piastrine giganti | Sangue |
IL RIPARO!

**INJURY**

Cellular and vascular response

- **Stimulus removed**
  - (acute injury)
  - Parenchymal cell death (intact tissue framework)
  - Superficial wounds
  - Some inflammatory processes
  - **REGENERATION**
    - Restitution of normal structure
    - Examples: Liver regeneration after partial hepatectomy, superficial skin wounds, resorption of exudate in lobar pneumonia

- **Persistent tissue damage**
  - Parenchymal cell death (damaged tissue framework)
  - Deep wounds
  - **REPAIR**
    - Scar formation
    - Examples: Deep excisional wounds, myocardium infarction
  - **FIBROSIS**
    - Tissue scar
    - Examples: Chronic inflammatory diseases (cirrhosis, chronic pancreatitis, pulmonary fibrosis)

*Kumar et al.: Robbins & Cotran Pathologic Basis of Disease, 8th Edition. Copyright © 2009 by Saunders, an imprint of Elsevier, Inc. All rights reserved.*
Fattori locali che influiscono sulla guarigione delle ferite

Infezione (può cronicizzare il danno tissutale)

Fattori meccanici (es. la mobilizzazione precoce può separare i lembi della ferita)

Corpi estranei (es. frammenti di acciaio, vetro e osso possono ostacolare la guarigione)

Dimensione, localizzazione e tipo di ferita (es. ferite in zone molto vascolarizzate come il volto guariscono più rapidamente di quelle in zone poco vascolarizzate come il piede)
Fattori sistemici che influiscono sulla guarigione delle ferite

Stato nutrizionale (es. carenza di vitamina C) **SCORBUTO**

Stato metabolico (es. diabete mellito)

Stato del circolo (es. aterosclerosi con ridotta perfusione o vene varicose con ridotto drenaggio venoso)

Ormoni (es. glucocorticoidi con azione anti-infiammatoria e inibente sulla sintesi del collagene)
La formazione dei cheloidi e di cicatrici ipertrofiche come esito del processo di riparazione

Shih B, Garside E, McGrouther DA, Bayat A..
Molecular dissection of abnormal wound healing processes resulting in keloid disease.
The dermis and the subcutis. A common characteristic is the increased deposition of collagen and proteoglycans within overgrown, but histologically do not extend beyond the differentiates them from hypertrophic scars that may appear organized. Keloids invade surrounding normal dermis, which proliferative to a static state with collagen that is increasingly morphology, with the hypertrophic scar phasing through a the precise characterization of the cell or tissue source (25). histopathological scar analysis is the correct sampling and within the entire scar tissue. Therefore, the major obstacle of point in time, these alterations do not occur simultaneously functional changes, which eventually can be identified at one pathological scarring is conditional on morphological and phases of keloids and hypertrophic scars (39). Although common matrix morphology and cellular function in the early diagnosis is regarded to provide the arbitration of highest results in a significant burden for the patient (8,9,37,38). Besides their variable extent of elevation, keloid scars can disfigurement not only leads to a cosmetic nuisance, but often the elevated tissue (4,11,30). Furthermore, the resulting keloid shows cessation of scar growth but no degeneration of levels of more than 0.5 cm.

### Table II. Scar classification.

<table>
<thead>
<tr>
<th>Scar type</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature scar</td>
<td>A light-colored, flat scar.</td>
</tr>
<tr>
<td>Immature scar</td>
<td>A red, sometimes itchy or painful, slightly elevated scar. Many of these will mature normally and become flat with a pigmentation similar to the surrounding skin.</td>
</tr>
<tr>
<td>Linear hypertrophic scar</td>
<td>(Surgical or traumatic anamnesis). A red, raised, sometimes itchy scar confined to the border of the original skin incision. Usual occurrence within weeks after surgery. Rapid increase in size for 3 to 6 months and then, after a static phase, regression. Full maturation process can take up to 2 years resulting in an elevated, slightly rope-like appearance.</td>
</tr>
<tr>
<td>Widespread hypertrophic scar</td>
<td>(Burn anamnesis). A widespread, red, elevated, sometimes pruritic scar confined to the borders of the burn injury.</td>
</tr>
<tr>
<td>Minor keloid</td>
<td>A focally raised, itchy scar extending over normal tissue. Possible development up to 1 year after injury and with no spontaneous regression. Surgical excision is often followed by recurrence.</td>
</tr>
<tr>
<td>Major keloid</td>
<td>Large, elevated (&gt;0.5 cm) scar, possibly painful or pruritic, extending over normal tissue. Spreading can continue over years.</td>
</tr>
</tbody>
</table>

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"Modified from Mustoe, 2002 (7)."
Excess scar formation occurs after dermal injury as a result of abnormal wound healing. Hypertrophic scars and keloids both represent fibrotic skin conditions which can be very difficult, even frustrating, to treat. Identification of differences between hypertrophic scars, keloids and normal scars are a prerequisite for finding the correct therapeutical concept. Despite the relatively high prevalence of keloids in the general population, the mechanisms underlying keloid formation are only partially understood. This fact is reflected in the multiple treatment modalities, of which no single treatment has proven to be widely effective. Advances in our understanding of the wound healing process reveal new pathophysiological concepts for keloid formation. Our article presents an overview on physiological wound healing and the pathogenesis of scar formation, differentiates keloids from hypertrophic scars and reviews current hypotheses for keloid formation. This information might assist in deciphering the complexity of keloid pathogenesis and help in the development of an efficacious therapeutical strategy.
relevant chronic illnesses, gender, gravidity, age, race, skin type or even lifestyle (sun exposure, smoker versus non-smoker). Major limiting factors during the healing process are tensile forces acting on the wound area or wound infection. Wound healing in adult human skin results in varying degrees of scar formation, ranging clinically from asymptomatic scars, to scars that are solely noticeable and problematic that are accompanied by functional confinement (29). Scarring, even in terms of non-pathological aberrances, remains an individual process which can be subdivided into a spectrum of nuances. As a result, a number of scar classification schemes have been established in plastic surgery literature describing main groups of wounds ranging from normal mature scars to major keloids, with linear and widespread hypertrophic scars placed somewhere near the middle (4,7,30) (Table II).

### 4. Keloids versus hypertrophic scars

A mature scar is light-colored, a flat fine line which only rarely entails functional deficits of the surrounding tissue. In most cases no therapy is required for functional reasons. As summarized in Table I, keloids and hypertrophic scars are separate clinical and histochemical entities (4,8,11,31). The first challenge to effective scar therapy must be taken seriously which is the correct identification and diagnosis of problem scars.

In cases of hypertrophic scars, the wound healing process begins with normal scarring, but the accumulation of repair matrix occurs for a longer phase, with increasing morphologic and biochemical abnormality. Hypertrophic scars are typically red or pink in color, often pruritic, elevated but remaining within the confines of the original wound, induced either by trauma or surgery (8,32). The first occurrence is usually several weeks after surgery. The time course is considerably prolonged in comparison to the normal scar cycle. Often it effects the scar as far as form and function are concerned, particularly when caused by contraction, which is worse than the effects in a mature scar (25). However, after a rapid increase in size, a static phase begins which spontaneously passes into a regression period (3,7). The maturation process of hypertrophic scars can take up to two years time. In contrast, keloid scarring does not follow the same pattern of evolution, stabilization and involution. Keloids may appear directly after an initiating event or start to grow some years later arising from a mature scar. The latter characteristic suggests some kind of activity within mature scar tissue, even though the relevant outside influences and the potential extent of alteration are not yet known. Keloids usually present in individuals between 10 and 30 years of age and are less frequent at the extremes of age (3). Although the keloid reaction is less intense than in hypertrophic scars, the continuous proliferation surpasses the growth of hypertrophic scars.

---

### Table I. The main differences between keloids and hypertrophic scars.

<table>
<thead>
<tr>
<th></th>
<th>Keloid</th>
<th>Hypertrophic scar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>Rare</td>
<td>Frequent</td>
</tr>
<tr>
<td>Associated skin type</td>
<td>Higher prevalence in dark-pigmented populations</td>
<td>None</td>
</tr>
<tr>
<td>Foregone skin injury</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Typical site</td>
<td>Everywhere; most frequently ear lobe, sternum</td>
<td>Everywhere</td>
</tr>
<tr>
<td>Spontaneous regression</td>
<td>None</td>
<td>Frequent</td>
</tr>
<tr>
<td>Recurrence after surgical excision</td>
<td>Almost 100%</td>
<td>None</td>
</tr>
<tr>
<td>Contracture</td>
<td>Seldom</td>
<td>Often</td>
</tr>
<tr>
<td>Expansiveness</td>
<td>Infiltrates adjacent normal dermis</td>
<td>Confined to wound tissue</td>
</tr>
<tr>
<td>Time relation of appearance</td>
<td>Appearance after symptom-free interval, proliferation without quiescent or regressive phase</td>
<td>Emergence within 4 weeks, intense grow for several months, then regression</td>
</tr>
<tr>
<td>Orientation of the collagen fibres</td>
<td>Hypocellular collagen bundles, fibres are larger, thicker, more wavy, random orientation</td>
<td>Parallel orientation to the epidermal surface</td>
</tr>
<tr>
<td>Myofibroblasts</td>
<td>None</td>
<td>Abundant nodules</td>
</tr>
<tr>
<td>Collagen</td>
<td>Increased ratio of type I to type III</td>
<td>Primarily collagen III</td>
</tr>
<tr>
<td>$\alpha(I)$-procollagen</td>
<td>Increased gene transcription and protein synthesis</td>
<td>Only increased mRNA concentration, compensation at the posttranscriptional level</td>
</tr>
</tbody>
</table>
Le cicatrici ipertrofiche e i cheloidi sono il risultato di un alterata risposta della cute ad un trauma cutaneo di varia natura, (chimico, fisico, chirurgico, infettivo) oppure, ma solo in soggetti predisposti, di origine spontanea.

I meccanismi molecolari dei cheloidi e delle cicatrici ipertrofiche

RUOLO DEL TGF-BETA?
I diversi fattori che portano alla formazione dei cheloidi

Shih B, Garside E, McGrouther DA, Bayat A..
Molecular dissection of abnormal wound healing processes resulting in keloid disease.
Alterazioni molecolari nei cheloidi

Shih B, Garside E, McGrouther DA, Bayat A..
Molecular dissection of abnormal wound healing processes resulting in keloid disease.
Molecular dissection of abnormal wound healing processes resulting in keloid disease.


Alterazioni della trasduzione del segnale nei fibroblasti dei cheloidi
Keloids are recognized as benign tumours characterized by fibroblastic proliferation and accumulation of extracellular matrix, especially collagen deposition. The transforming growth factor (TGF)-β1/Smad pathway plays an important role in keloid pathogenesis; however the underlying mechanisms are not fully understood.

The results of this study suggested that the ERK, JNK and p38 pathways mediate TGF-β1/Smad signal transduction and might be considered as specific targets of drug therapy for keloids.
Despite their benign nature, keloids are usually associated with considerable cosmetic effects and may lead to functional problems. Recently, it has been reported that vascular endothelial growth factor (VEGF), a potent angiogenic factor, is overexpressed in keloid tissue and may have a potential role in its evolution.

Marked histopathologic changes after therapy were associated with the highest decrease in VEGF expression, particularly in patients treated by intralesional steroids.

Before treatment: showing strong staining reaction for VEGF (DAB ×400).

After treatment: showing weak staining reaction for VEGF (DAB ×400).

VEGF seems to play an important role in the pathogenesis of keloids and may be a useful guide in the evaluation of keloid therapeutics. Modulation of its production may provide a valuable treatment for keloids.
receptor is the integrin α5β1 which is also known to be expressed by fibroblasts (79). Integrin expression is regulated by cytokines and growth factors released from the ECM through limited proteolysis or from adjacent cells by autocrine and paracrine mechanisms (9,37,78). On fibroblasts, collagen recognition is mainly achieved by α1β1 and α2β1 integrins. Several findings suggest that binding of α1β1 integrin to collagen I results in an almost complete arrest of collagen synthesis according to a negative feedback regulation mechanism (80). Antibody blockage of α1β1 integrin prevents downregulation of collagen synthesis (81). Binding of α2β1 integrin has no effect on collagen synthesis (77). An abnormal reduction in α1β1 expression could result in a loss of the negative feedback which could explain the increased collagen synthesis within keloid tissue. However Szulgit et al (79) observed the greatest expression of α1β1 integrin in keloidal fibroblasts. This finding might be due to the influence of the profibrotic cytokine TGF-β which is found in elevated concentrations in the ECM of keloids, and has been shown to upregulate surface integrin expression (82). So far little research has been conducted on the expression of integrins within keloidal lesions. Further study is required to understand the correlation between the ECM milieu and the maintenance and function of fibroblast integrin expression.

An essential feature of tissue repair and remodeling processes is the proteolytic degradation of the ECM. Within the remodeling phase during wound healing, collagen III is substituted by collagen I, proteoglycans are synthesized and fibrin and fibronectin are degraded. The two major groups of ECM-degrading enzymes are the serine proteinases, including tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), and the matrix metalloproteinases (MMPs). They interact and form a lytic cascade for ECM remodeling (9). MMP-1 (collagenase-1), -8 (collagenase-2) and -13 (collagenase-3) degrade collagen types I, II and III by proteolysis of the triple helix (83). The major function of the plasminogen activator is the control of activation of plasminogen into plasmin. Plasmin is the primary effective enzyme in fibrinolysis. It also activates procollagenase into collagenase and participates in other breakdown processes of ECM proteins. The initiation of the proteinase cascade by plasminogen activator results in an important amplification of proteolytic activity (9). The complexity of the entire system can be visualized by the control feedback loop of plasmin, which also induces the release of active TGF-β from its latency-associated protein (84). The released TGF-β regulates plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinases, tissue inhibitor proteinases-1 (TIMP-1) and genes encoding ECM components and their integrin receptors (9).

The formation of the extracellular matrix is carried out by the synthesis of collagen, fibronectin and proteoglycans, all conducted by fibroblasts (31). A deficient synthesis of products that promote matrix degradation or an excessive matrix synthesis, or both, explain the lack of scar regression in keloids (3,85). Collagen degradation is mediated by MMPs. TGF-β modulates the expression of MMPs (86). TGF-β is known to induce the expression of MMP-2, -9 and -13 in fibroblasts, whereas MMP1 expression is negatively regulated through SMAD 3 and 4 (87). The extracellular matrix of keloids shows elevated levels of fibronectin and proteoglycans as well as MMPs. Neely et al reported significantly increased MMP-2 activity in keloids and no change in MMP-9 activity (88). Besides elevated levels of MMP-1, -2 and TIMP-1, BRAN et al: PATHOGENESIS OF KELOIDS

Figure 5. The relaxed skin tension lines correspond to the skin folds of an aged face (left) and run perpendicularly to the facial musculature (right).
During physiological wound healing, mechanical tension accumulates within the contracting wound site. Mechanical tension on a healing wound stimulates fibroblast proliferation and increases the synthesis and deposition of collagen, citing sites of keloid predilection in areas of increased tension (e.g. chest, deltoid and back) (32,71-73). Skin tension could be the reason why keloids occur in young people and are almost absent in the elderly, whose skin characteristically has poor tension (32,68). Calnan and Copenhagen observed a regression of keloid tissue after autotransplantation of keloids to the anterior abdominal wall, a site of little wound tension (74).

In vitro and in vivo studies suggest that mechanical strain not only promotes collagen synthesis but also dictates collagen architecture and orientation as well as dermal remodeling (75). The physiological orientation of collagen is perpendicular to muscle contraction. Therefore, incisions performed perpendicular to the muscle contraction, and parallel to the relaxed skin tension lines, will heal with no or little collagen distortion (Fig. 5), whereas non-aligned tension forces due to an inadequate choice of incision line or scars placed at sites of high tension (e.g. flexor surfaces) will very likely result in pathologic scar formation (8). Wang et al compared the effects between normal and keloid fibroblasts subjected to mechanical strain. They observed an increased expression in TGF-β1, -β2, and collagen I in keloid fibroblasts. Additionally, there was increased formation of focal adhesion complexes with increased activation of focal adhesion kinase, a signaling component of the focal adhesion complex (76).

However, sites of frequent keloid formation (e.g. earlobe or chest) which are not generally accepted to be under tension also exist (32). Although stretch and tension are important parameters of the final appearance of the scar, they are more likely to play a role in hypertrophic scar formation than they do in keloid formation (8,75).

Figure 4. Keloid formation after anthelixplasty (A) or piercing of the ear lobe (B). Histological picture of a keloid (C) with enlarged collagen fibres within the dermis (D).
DAGLI EGIZI........

Sh-m-m-t:
Geroglifico di infiammazione e riparo
alla copertina di times del 23 febbraio 2004
...al fatto che l'infiammazione è un potente meccanismo che il nostro organismo attiva per segnalarci che è in atto un danno da riparare!!!