Progetto Ematologia Romagna

La diagnostica dell'infiammazione

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Infiammazione

Meccanismo protettivo in risposta a
danno tissutale dovuto a infezioni, trauma, agenti chimici

↓

citochine infiammatorie

↓

I granulociti neutrofili sono le prime cellule a raggiungere il sito infiammatorio seguendo il gradiente di citochine infiammatorie;
Infiltrato immune di linfociti e macrofagi

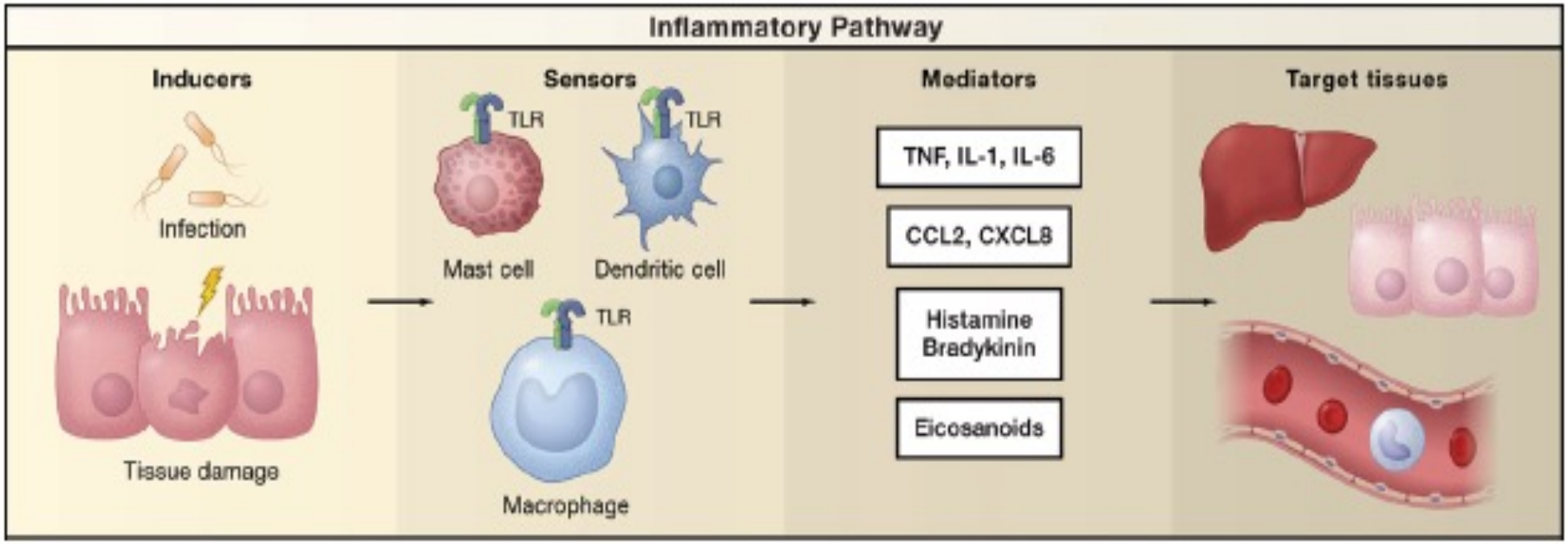
Tipica risposta infiammatoria:

1)Induttori infiammazione

2)Sensori infiammazione

3)Mediatori dell'infiammazione

4)Tessuto target



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Infiammazione/Infiammazioni?

Tipo infezione:

Batteri
Virus
Parassiti

Sensori
Mediatori
Tessuto target

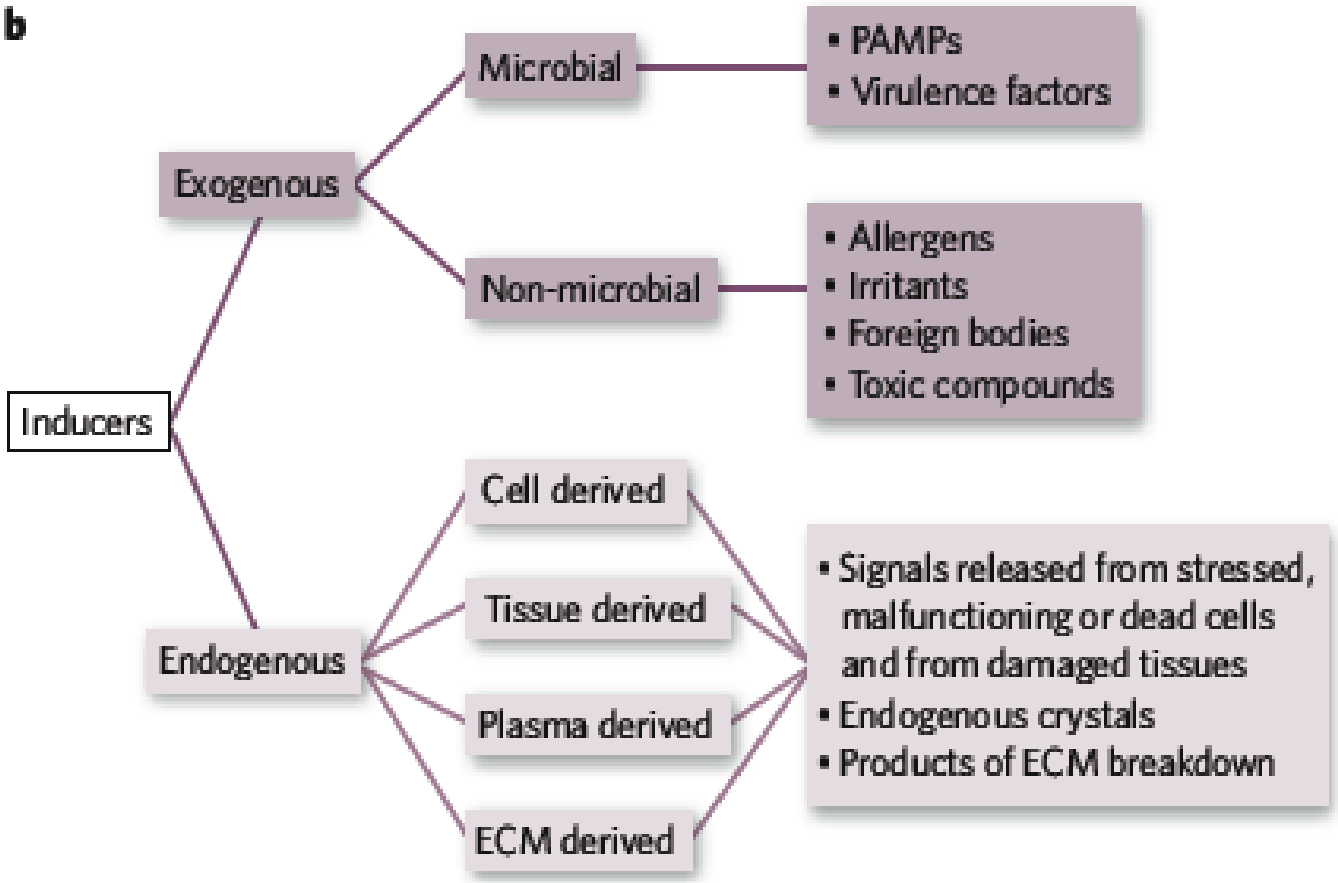
⇩

differenti

⇩

appropriata risposta infiammatoria

INDUTTORI DELL'INFIAMMAZIONE



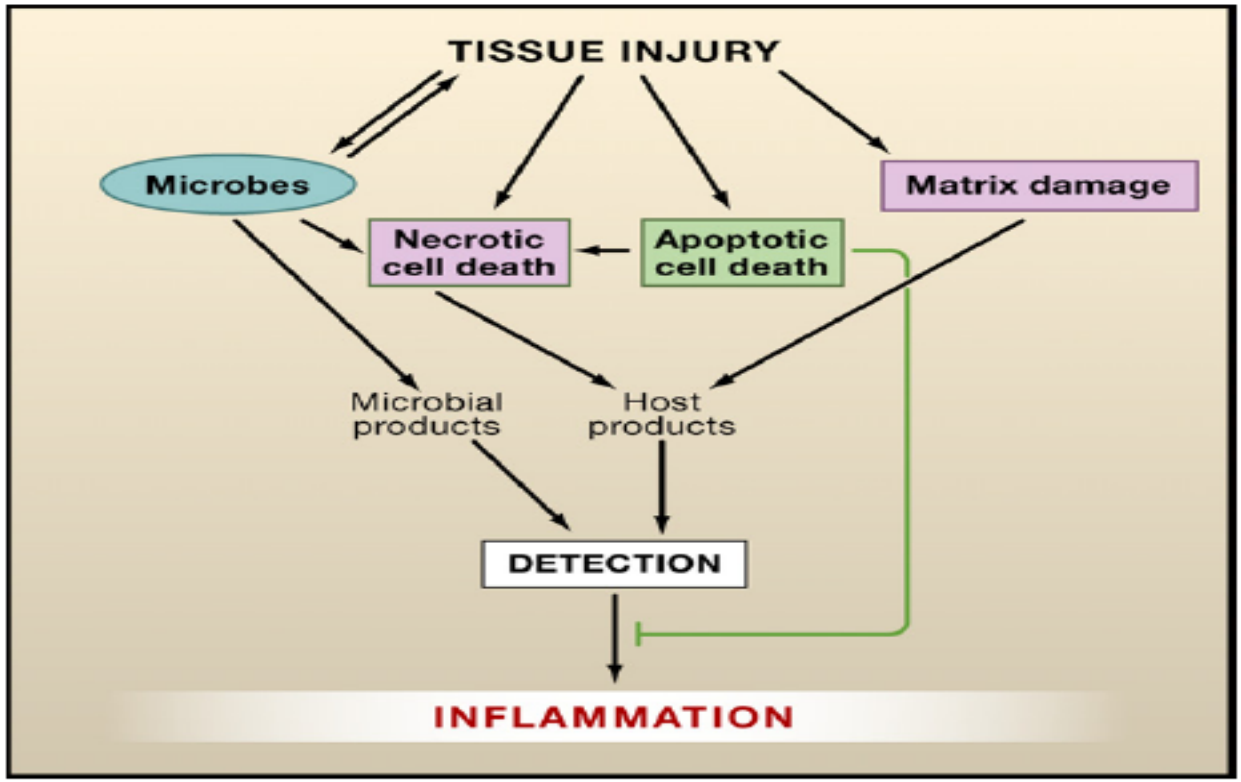
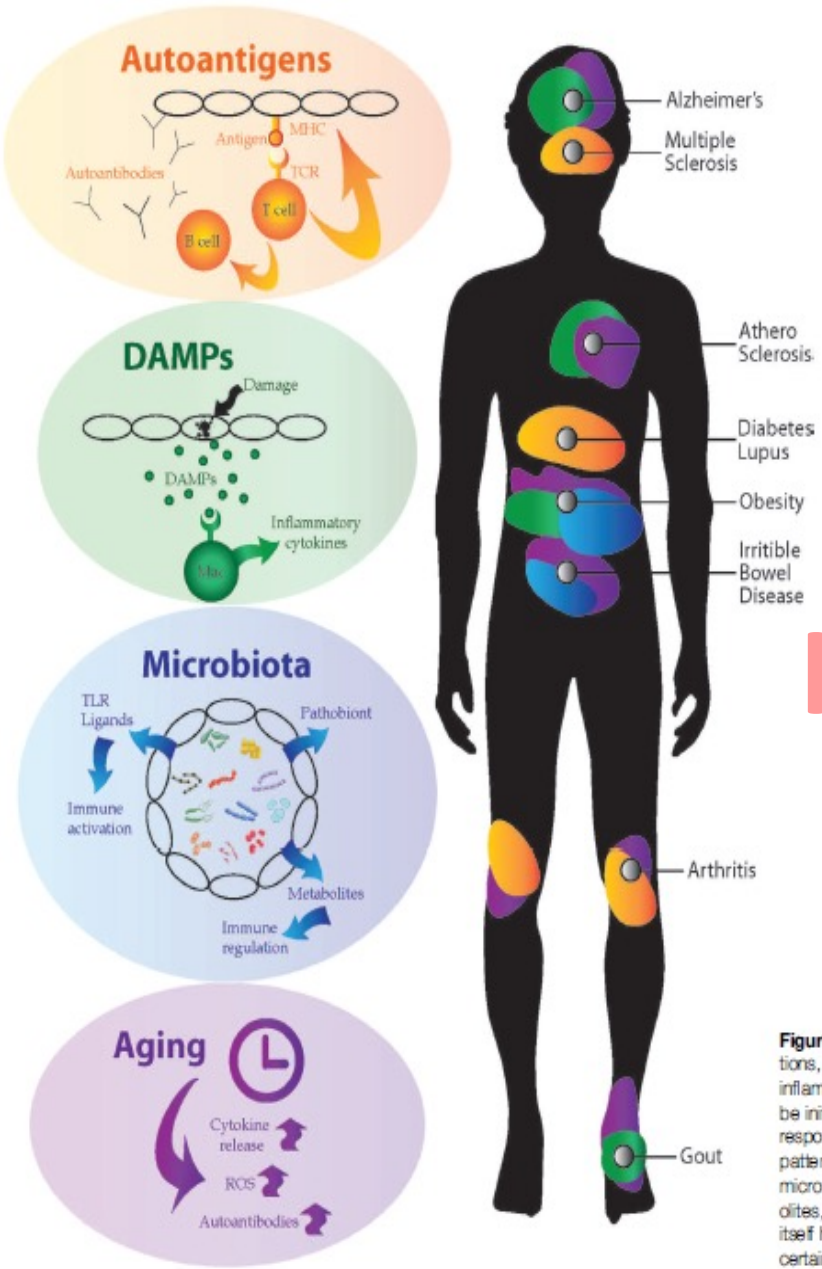


Figure 2. Initiation of Prolonged Inflammation Usually Requires Signals from Both Microbes and Injured Tissue

Cell *140*, 871–882, March 19, 2010 ©2010 Elsevier Inc. 871



INFIAMMAZIONE CRONICA

Figure 1. Mechanisms, anatomical locations, and disease types involving chronic inflammation. Chronic inflammation can be initiated by autoantigens or in response to damage associated molecular patterns (DAMPs). Furthermore, the microbiota – via the action of their metabolites, etc. – as well as the aging process itself have been shown to be involved in certain types of chronic inflammation.

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Biomarkers infiammatori, quale/i dovrebbe(ro) essere raccomandato/i?

Infiammazione: diagnostica

OLD

- **Citochine**
- **Proteina C reattiva**
  Fibrinogeno
  N° globuli bianchi

NEW

- **MicroRNA**
- **Microbiota**

Citochine

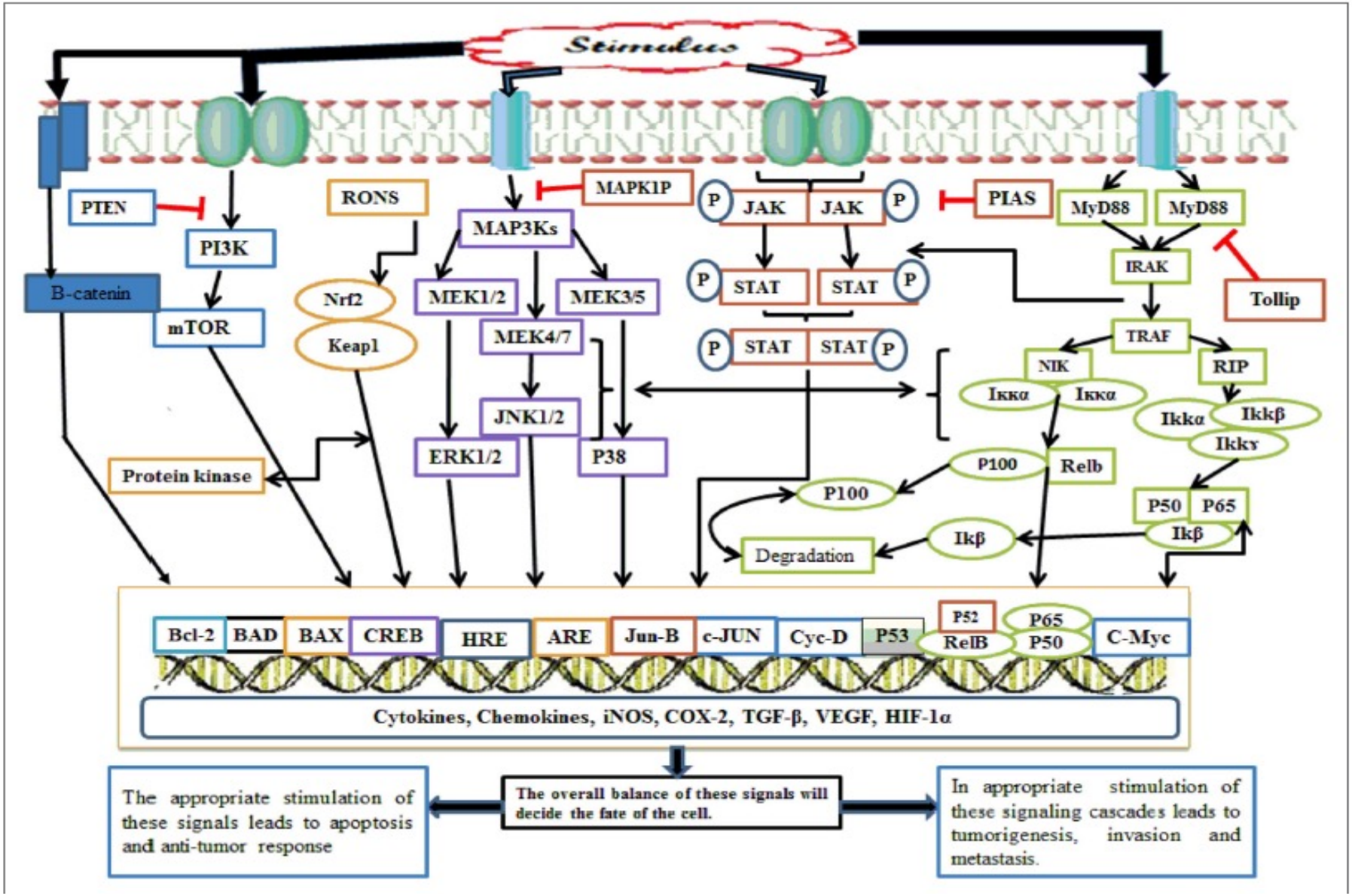
Molecole di segnale coinvolte nell'infiammazione e sintetizzate in risposta a stimoli del microambiente

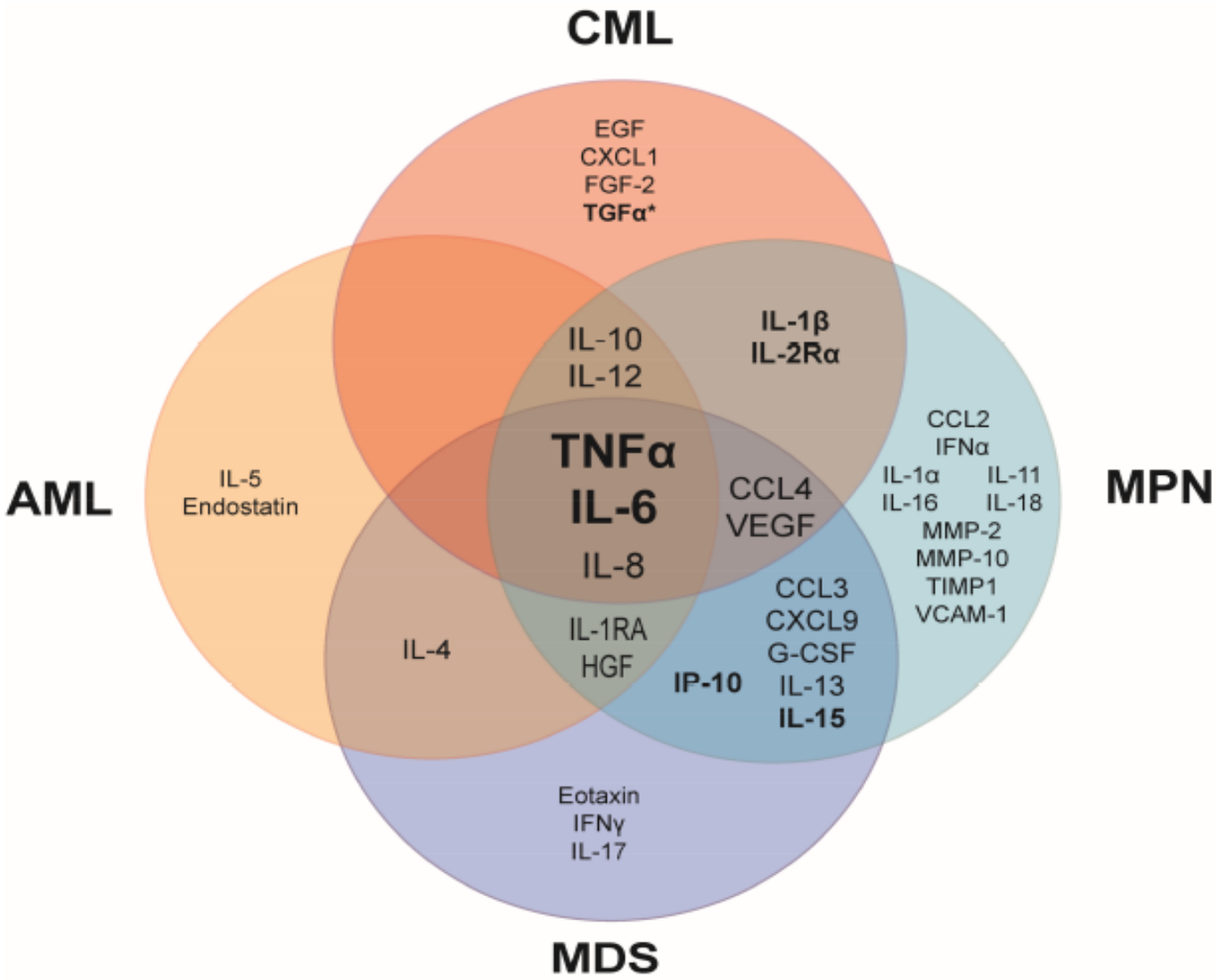
Classicamente in 2 classi:

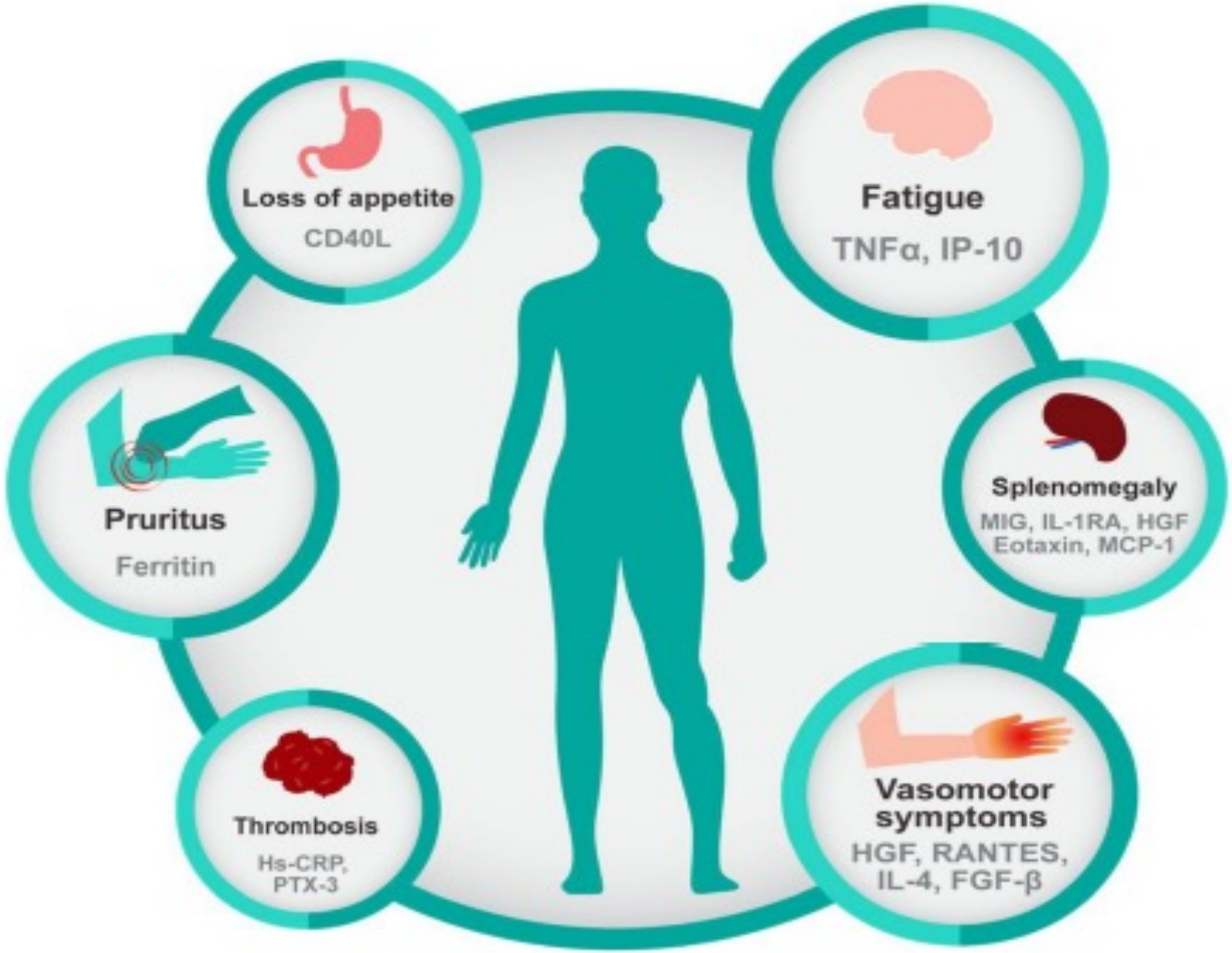
Citochine anti-infiammatorie: IL-4, IL-10, IL-13, TGF-β

Citochine infiammatorie: IL-1β, IL-6, IL-15, IL-17, IL-23; TNF-α

"signalling intracellulare": NF-KB/MAPK/JAK-STAT


Stimulus
PTEN
PI3K
B-catenin
mTOR
RONS
Nrf2
Keap1
MAPK1P
MAP3Ks
MEK1/2
MEK3/5
MEK4/7
JNK1/2
ERK1/2
P38
Protein kinase
JAK
JAK
STAT
STAT
STAT
STAT
PIAS
MyD88
MyD88
IRAK
Tollip
TRAF
NIK
RIP
Ikkα
Ikkα
Ikkα
Ikkβ
Ikkγ
P100
Relb
P100
Degradation
Ikβ
P50
P65
Ikβ
Bcl-2
BAD
BAX
CREB
HRE
ARE
Jun-B
c-JUN
Cyc-D
P53
P52
RelB
P65
P50
C-Myc
Cytokines, Chemokines, iNOS, COX-2, TGF-β, VEGF, HIF-1α
The appropriate stimulation of these signals leads to apoptosis and anti-tumor response
The overall balance of these signals will decide the fate of the cell.
In appropriate stimulation of these signaling cascades leads to tumorigenesis, invasion and metastasis.


CML
EGF
CXCL1
FGF-2
TGFα*
IL-10
IL-12
IL-1β
IL-2Rα
AML
IL-5
Endostatin
TNFα
IL-6
IL-8
CCL4
VEGF
CCL2
IFNα
IL-1α
IL-11
IL-16
IL-18
MMP-2
MMP-10
TIMP1
VCAM-1
MPN
IL-4
IL-1RA
HGF
CCL3
CXCL9
G-CSF
IP-10
IL-13
IL-15
Eotaxin
IFNγ
IL-17
MDS


Loss of appetite
CD40L
Fatigue
TNFα, IP-10
Pruritus
Ferritin
Splenomegaly
MIG, IL-1RA, HGF
Eotaxin, MCP-1
Thrombosis
Hs-CRP,
PTX-3
Vasomotor
symptoms
HGF, RANTES,
IL-4, FGF-β

Proteina C reattiva

- **Marker di infiammazione non specifico:**
  aumento in risposta a danno tissutale, infezione, infiammazione
- **Sintetizzata dal fegato in risposta ad elevati livelli di IL-6, IL-1 e TNF**
- **Ha un' emivita di circa 19 ore in circolo**
- **Valore predittivo eventi cardiovascolari**

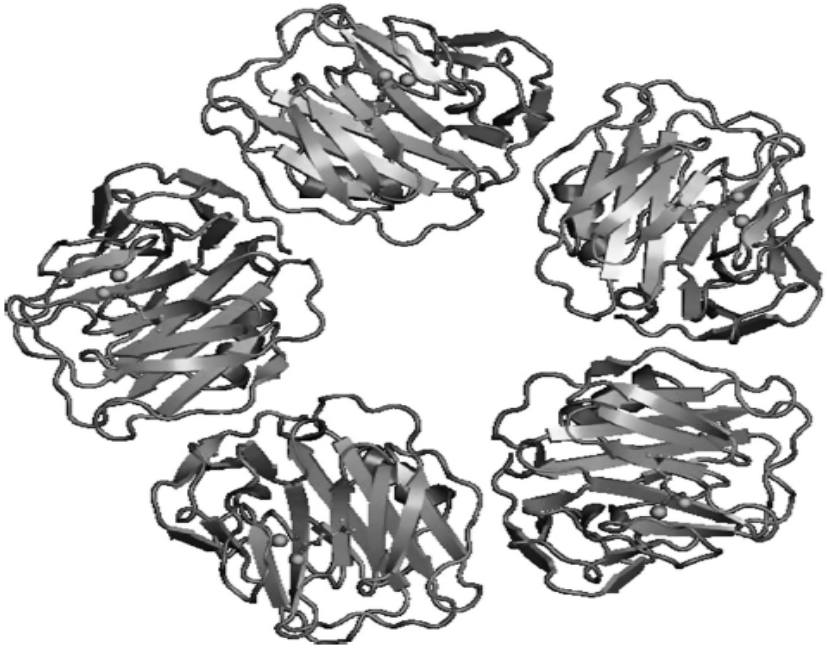


Figure 1 Pentameric structure of CRP.[27]

pCRP/mCRP

Ruolo distinto nel processo infiammatorio

pCRP ruolo anti-infiammatorio
mCRP ruolo pro-infiammatorio

Proteina C reattiva

- **Ruolo principale: attivazione C1q con opsonizzazione patogeno/ ruolo protettivo contro le infezioni batteriche/livelli circolanti**
  Non distinguono tipo di infezione
- **Non solo marker di infiammazione ma ruolo attivo nell' infiammazione:**
  Legame a recettori Fc con induzione rilascio citochine infiammatorie
- **Ruolo nell' apoptosi:produzione citochine pro-apoptotiche/ promuove opsonizzazione cellule apoptotiche e fagocitosi**
- **NON SOLO MARKER INFIAMMAZIONE/INFEZIONE**
  IMPORTANTE REGOLATORE INFIAMMAZIONE

Measurement of CRP

Standard and high sensitivity assays:

Traditional CRP assay, using a polyclonal antibody, assists in the diagnosis and assessment of acute inflammation, i.e., associated with infections and neoplastic diseases.

A more sensitive CRP test, the highly sensitive CRP assay, allows detection of CRP levels down to and below 3 mg/L. These lower levels reflect low-grade inflammation and have a predictive value of future risk for CVD events.

The relative cardiovascular risk categories for average hs-CRP levels are:

- low risk <1.0 mg/L
- average risk 1.0–3.0 mg/L
- high risk 3.0–10.0 mg/L
- unspecific elevation >10 mg/L (should be reevaluated for acute inflammatory conditions)

CRP / High sensitivity-CRP (hs-CRP) assays

Table 1
Acute phase inflammatory proteins and outcomes of MPN.

Author	Setting	Total patients	Biomarkers	Main results
Barbui 2011 [56]	ET (n = 173) and PV (n = 71) patients	244	hs CRP and PTX-3	PTX-3 and hs-CRP values were significant correlated with *JAK2*V617F allele burden greater than 50%. Patients with increased levels of hs-CRP (>3 mg/L) had an increased risk of major thrombosis. Conversely, high PTX-3 levels (>4.5ng/ml) decreased the rate of thrombosis
Barbui 2013 [57]	PMF (n = 167) and post PV or ET MF (n = 17)	184	hs CRP	Higher hs-CRP levels (≥7mg/dl) were independently associated with shortened leukemia free survival. Also the annual incidence rate of death was increased in patients with higher levels of hs-CRP.
Lussana 2017 [58]	ET (n = 305) and PV (n = 172) patients	477	hs CRP and PTX-3	Circulating levels of PTX-3 were significantly increased in homozygous *JAK2*V617F mutation carriers compared to all the other genotypes. The risk of hematological evolution and death from any cause was significantly increased in individuals with high PTX-3 levels, while high levels of PTX3 were associated with a trend to a lower risk of thrombosis. Patients with high hs-CRP levels exhibited an increased risk of hematological evolution and death and to a lower extent also of thrombotic events
Barosi 2017 [59]	PMF	526	hs-CRP	Subjects with JAK2V617F mutation and an allele burden ≥ 50% had an age-independent higher incidence of elevated hs-CRP level. ASXL1, EZH2 sub-clonal mutations, JAK2 46/1 haplotype and the A3669G polymorphism of glucocorticoid receptor were not significantly associated with increased hs-CRP levels.

PMF = primary myelofibrosis; ET = essential thrombocythemia; PV = polycythemia vera; PTX-3 = pentraxin 3; hs-CRP = high sensitivity C-reactive protein.

Infiammazione: diagnostica

OLD

- **Citochine**
- **Proteina C reattiva**
  Fibrinogeno
  N° globuli bianchi

NEW

- **MicroRNA**
- **Microbiota**

microRNA

Reprimono espressione genica
degradazione mRNA target/inibizione traduzione proteina

miR146a	repressore infiammazione
miR155	attivatore infiammazione
miR 181	attivatore infiammazione

miR circolanti :
esosomi o cell free (Risc complex)

Table 1. Selected examples of ncRNAs with roles in regulating inflammation

Species	Type	Disease	Cell types	Targets	Reference
miR-155	miRNA	CVD, viral infection, MS, RA, SLE, tumor immunity, chronic low-grade inflammation	Tfh, Th17, Th1, Th2, Macs, B cells, Treg, DCs	SHIP1, SOCS1, BACH1, PU.1, JARID2, PELI1, FOSl2, ETS1	[62, 63, 66–70, 77–79, 88, 106–108]
miR-146a	miRNA	Autoimmunity, dermatitis, chronic low-grade inflammation	Th1, Tfh, Treg, B cell, Macs, DCs, HSC	TRAF6, IRAK1, STAT1	[61, 68, 86, 89, 108, 109]
miR-17∼92	miRNA	Tumor immunity asthma, MS, viral Infection	Tfh, Th17, Th1, Th2, Treg, B cell	PTEN, PHLPP2, SOCS1, RORA, A20, IKZF4	[65, 80–82, 110, 111]
miR-181a	miRNA	Autoimmunity, aging-related inflammation	T cells	DUSP6, SHP2, DUSP5, PTPN22	[64, 112]
miR-182	miRNA	Tissue inflammation	T cells	FOXO1	[113]
miR-29a	miRNA	Crohn's disease	Th1, DC	TBET, EOMES, IL-12p40	[114, 115]
miR-125	miRNA	IBD, SLE	Macs	KLF13, IRF4	[71, 72]
miR-223	miRNA	Inflammatory lung pathology	Macs, granulocytes	Mef2c, Pknox1	[73, 74]
miR-124	miRNA	Neuro-inflammatory	Microglia	C/EBP-α, PU.1	[116]
LincRNA-Cox2	LncRNA	–	Macs	CCL5, IL-6	[117]
NeST	LncRNA	Microbial infection	T cells, NK cells	IFNG	[118]
LncDC	LncRNA	–	Macs	STAT3 target genes	[119]
CCR2	LncRNA	–	TH2	TH2 genes	[120]
E330013P06	LncRNA	Diabetes	Macs	–	[103]
Thril	LncRNA	Kawasaki disease	Macs	TNFα, IL-8, CXCL10, CCL1, CSF1	[121]

DC, dendritic cells; HSC, hematopoietic stem cell; Macs, macrophages; NK cell, natural killer cell; Tfh, T follicular helper cells; Tregs, regulatory T cells.

microRNA

microRNA regolano l'espressione di mRNA per citochine infiammatorie,
ma anche il "signalling" citochinico ha impatto sull'espressione
dei microRNA: IL-1β e TNF-α potenti stimolatori microRNA 155 e 146a

Biomarkers prognostici,
Biomarkers di conferma di un disordine
Biomarkers di risposta ad un determinato trattamento

In circolo associati a
1)Microvescicole
2) Complessi proteina/lipoproteina

Aberrante espressione microRNA associata a disordini infiammatori/autoimmuni

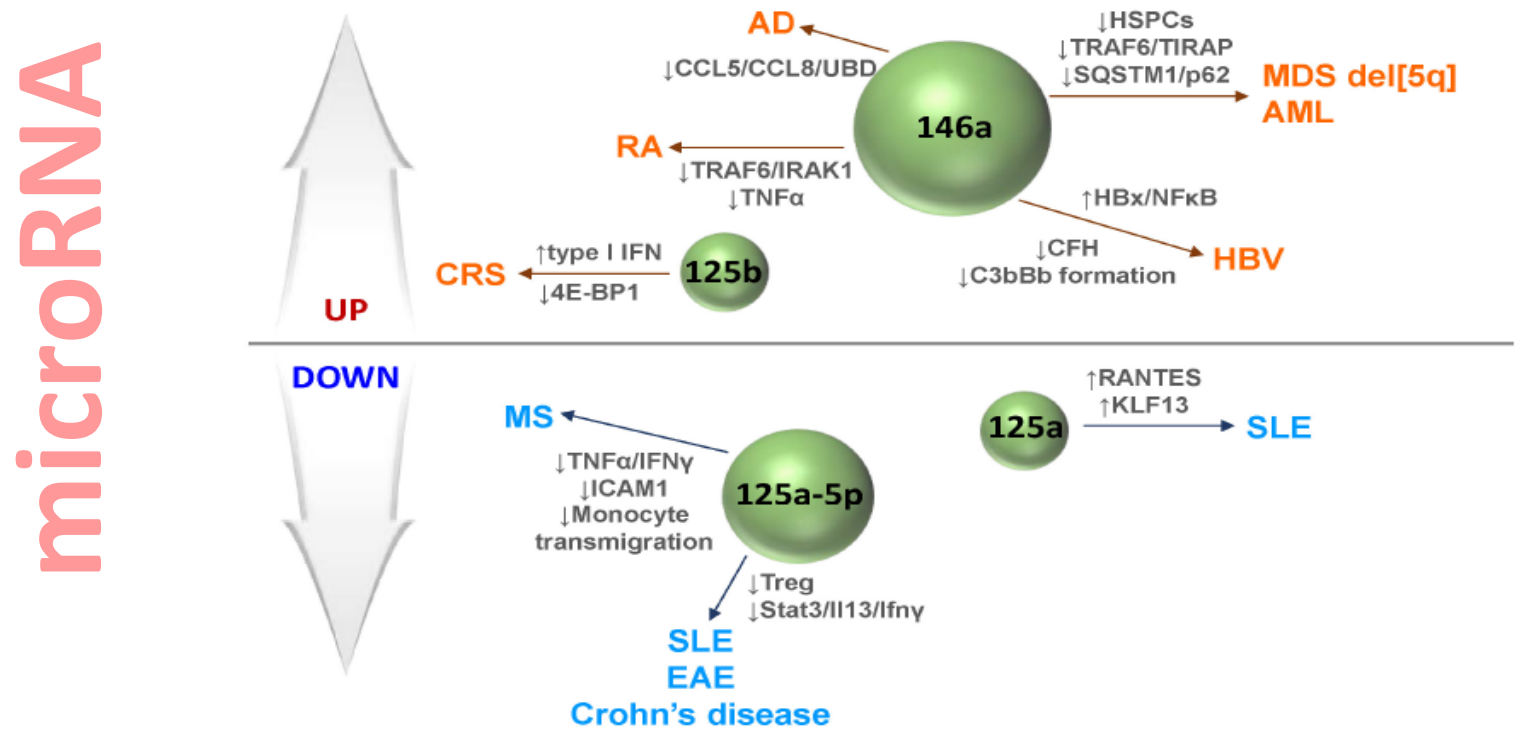
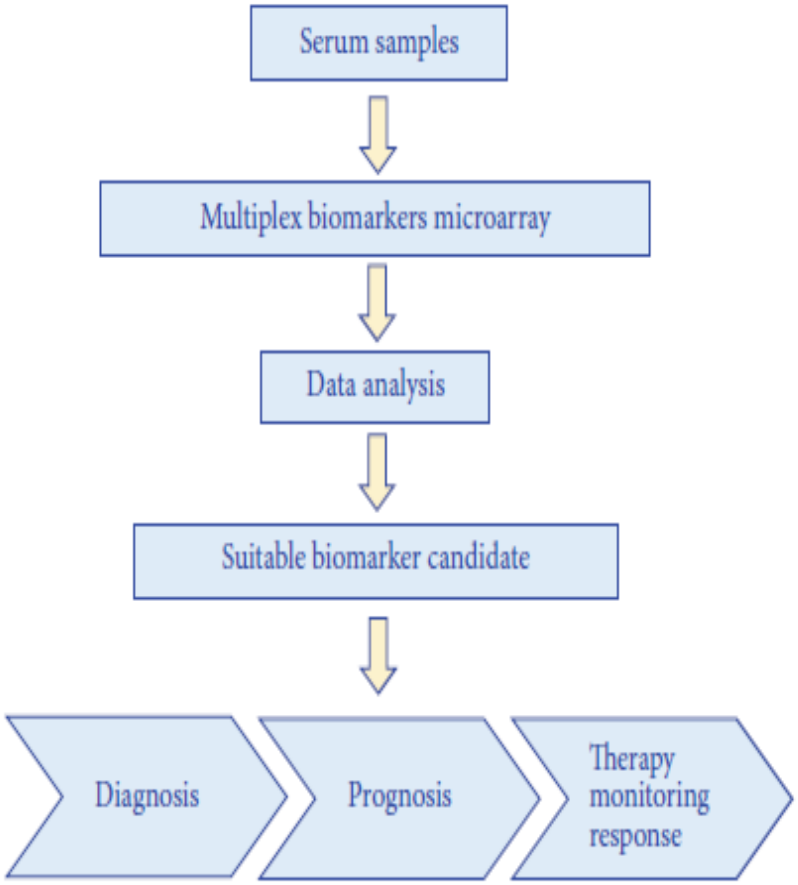
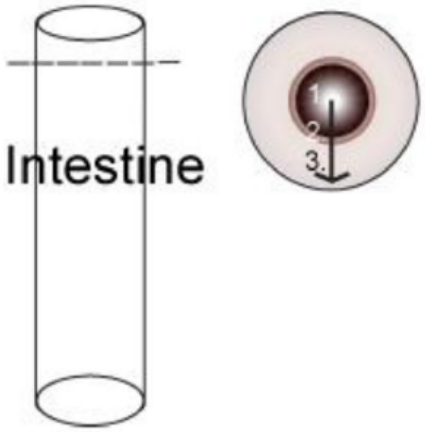
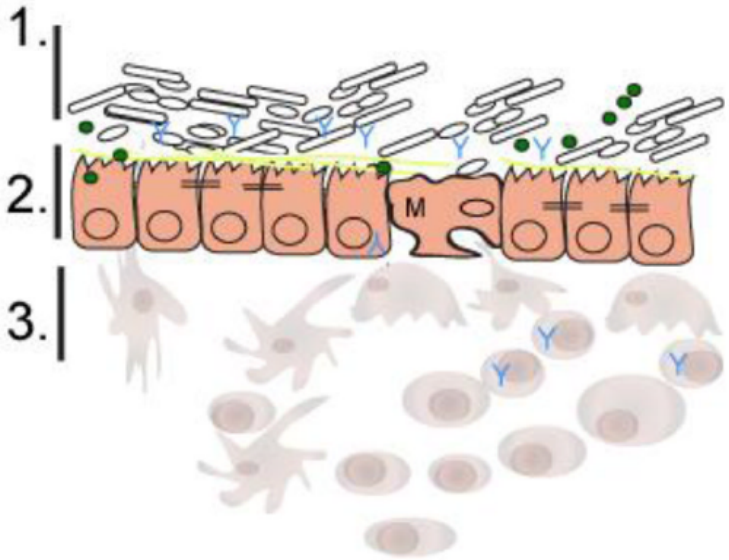


Fig. 3. Dysregulated levels of miR-125 and miR-146 in various infectious and inflammatory diseases. MiR-125 and miR-146 levels are upregulated or downregulated in various infectious and inflammatory diseases. Upper panel: up-regulated miRNA mediates chronic rhinosinusitis (CRS), Rheumatoid arthritis (RA), atopic dermatitis (AD), Myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and Hepatitis B virus infection (HBV). Lower panel: down-regulated miRNA mediates multiple sclerosis (MS), systemic lupus erythematosus (SLE), experimental autoimmune encephalomyelitis (EAE), systemic lupus erythematosus (SLE), and Crohn's disease.


Serum samples
Multiplex biomarkers microarray
Data analysis
Suitable biomarker candidate
Diagnosis
Prognosis
Therapy monitoring response

Microbiota





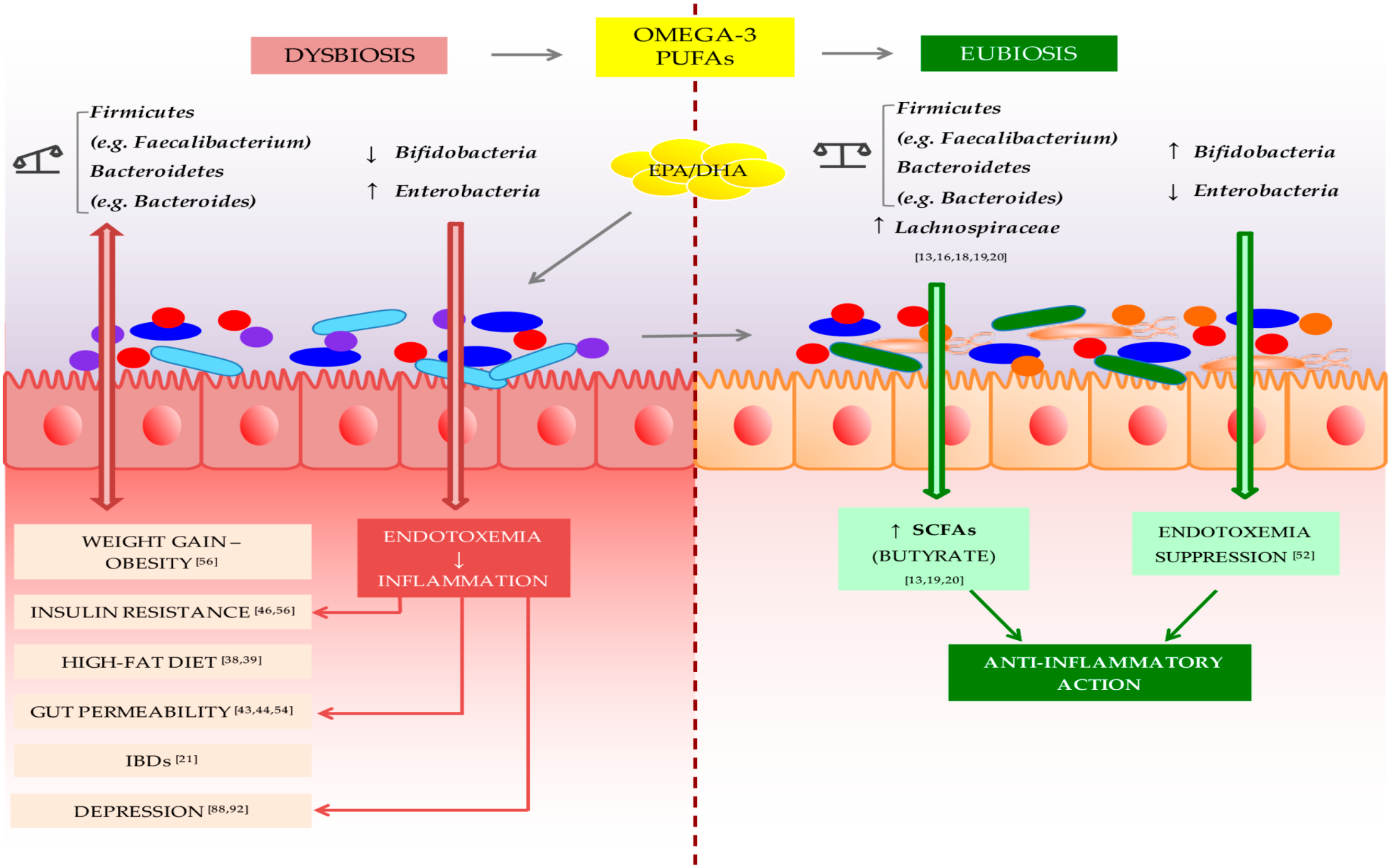
-100 trillioni di microbi nel corpo umano

-Immensa diversità filogenetica

-2 Phyla: Firmicutes e Bacteroidetes

-Elevata plasticità (dieta/farmaci/età)

-Funzione principale: immunomodulazione


DYSBIOSIS
OMEGA-3 PUFAs
EUBIOSIS
Firmicutes
(e.g. Faecalibacterium)
Bacteroidetes
(e.g. Bacteroides)
↓ Bifidobacteria
↑ Enterobacteria
EPA/DHA
Firmicutes
(e.g. Faecalibacterium)
Bacteroidetes
(e.g. Bacteroides)
↑ Lachnospiraceae
[13,16,18,19,20]
↑ Bifidobacteria
↓ Enterobacteria
WEIGHT GAIN – OBESITY [56]
ENDOTOXEMIA ↓ INFLAMMATION
INSULIN RESISTANCE [46,56]
HIGH-FAT DIET [38,39]
GUT PERMEABILITY [43,44,54]
IBDs [21]
DEPRESSION [88,92]
↑ SCFAs (BUTYRATE) [13,19,20]
ENDOTOXEMIA SUPPRESSION [52]
ANTI-INFLAMMATORY ACTION

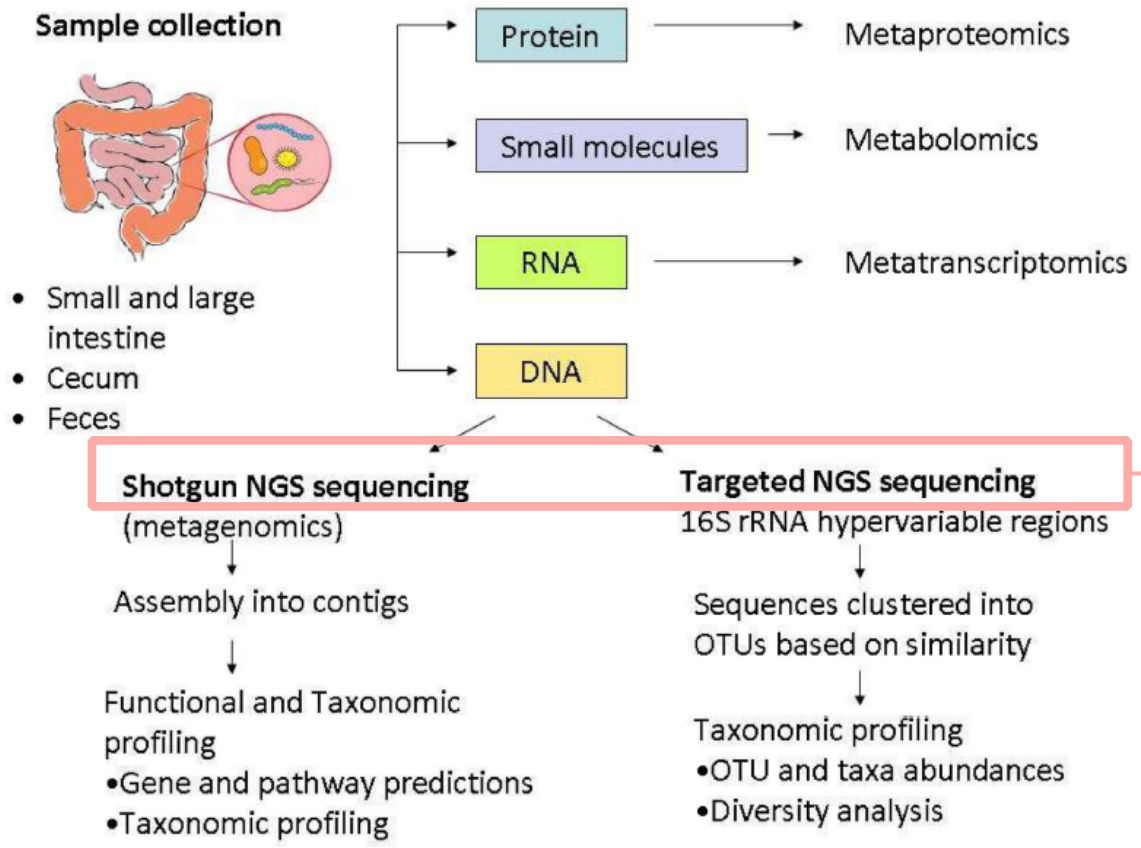


Figure 2. Overview of culture-independent methods for microbial analysis
High-throughput analysis of gastrointestinal tract microbiota via sequencing of DNA using targeted or shotgun sequencing or using other "omics" approaches. NGS, next generation sequencing; OTU, operational taxonomic units.

Diversità filogenetica

↓

Disbiosi

↓

Infiammazione

Conclusioni:

-Una infiammazione/tante infiammazioni
-PCR marker di infiammazione più studiato
-Nuovi markers (microRNA e microbiota) da validare

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