Metagenomes* of blood and psoriatic skin. Research project.

Presentation and illustrations. Supplement A. e2.2.

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Section 1.

SIBO (Small intestine bacterial overgrowth) at psoriatic disease.

Presumed Y-antigen and peptides.
PsB - bacteria presumed psoriagenic.

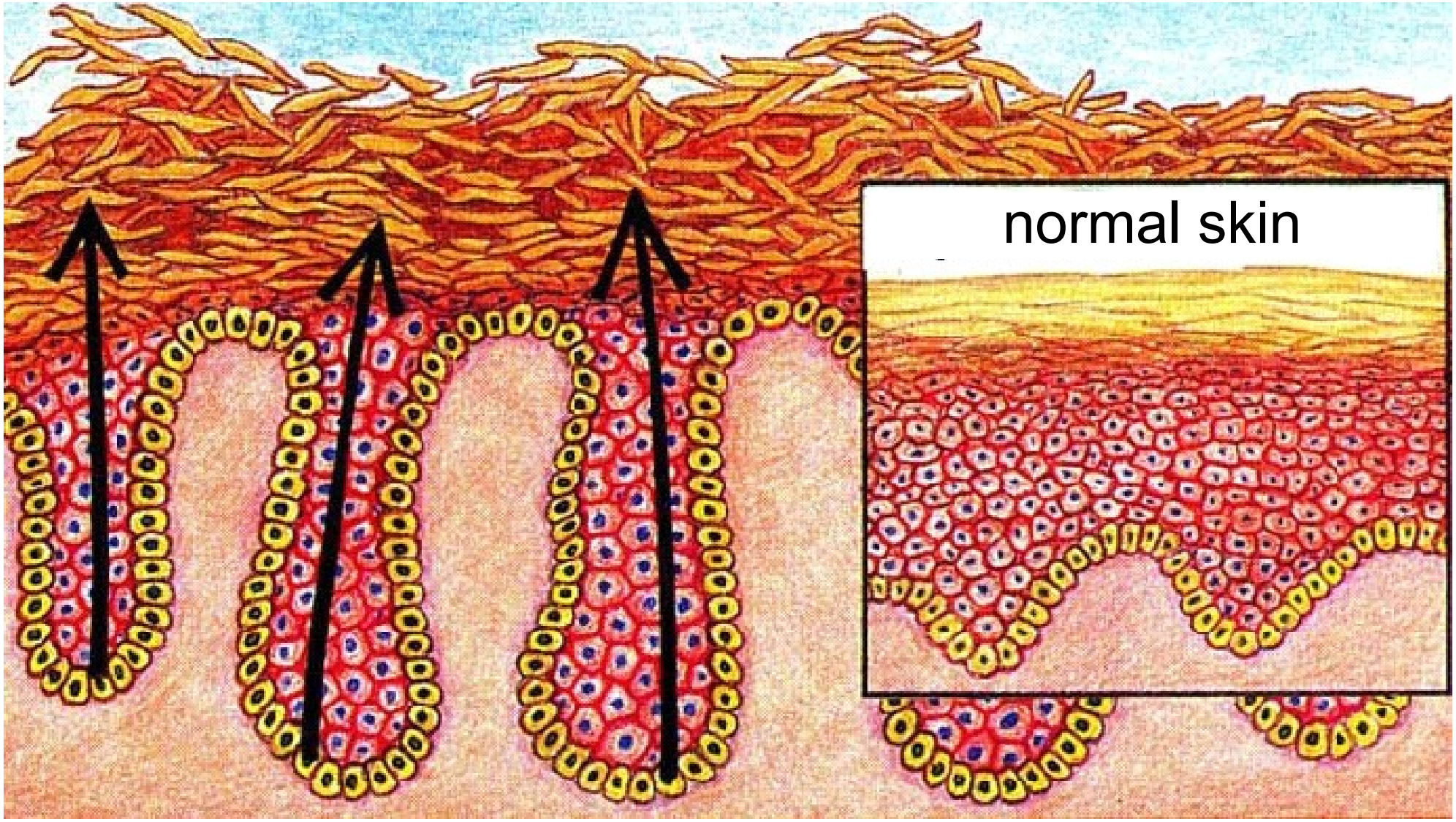
Systemic models of psoriasis pathogenesis
(BF-model, Y-model and YN-model).

Systemic psoriatic process SPPN and checked hypotheses.

* Metagenome is a complex of all nhDNA (non-host DNA, i.e. non-human here) contained in biomaterial. nhDNA is bacterial, archean, fungal, helminthic, viral, phage, etc. DNA.

The book, DOI of edition e2.2: 10.5281/zenodo.2667680
This presentation, DOI of edition e2.2: 10.5281/zenodo.2668376
This presentation, DOI of latest edition: 10.5281/zenodo.2668375

Psoriatic and normal skin



Growth of dermal papillae height leads to an increase in thickness of dermo-epidermal area. Arrows show direction of intensive proliferation of epidermal cells.

Statistics of PD incidence on countries

Country	Years	Number of examined	% with PD	Years	Patients in year on 100 000
China	1984	6 617 917	0.12		
China, Taiwan	2006	23 000 000	0.24		
China	1974–1981	670 000	0.35		
Germany	2005	1 344 071	2.53		
Germany	2003	2 238 000	2.0		
Italy	2006	4 109	2.9	2005	230 #
Japan	2010–2011	128 000 000	0.44		
Norway	1985	10 576	1.41		
Poland	2005–2009	2 161 832	1.45		
Portugal	1994	1 037	1.9		
Russia*	2004		~2 - 4	2009-13	216
Spain	1998	12 938	1.43		
Spain	2013	12 711	2.31		
Sweden	1998–2010	–	1.95		
UK	2009	7 520 293	1.87		
UK	1987–2002	7 533 475	1.52	1996-7	140
USA	1971–1974	20 749	1.43	1991	60
USA	2004	27 220	2.2	1970-2000	78,9 #
USA	2009	2 573	5.1		

*
Mishina O. S. Psoriasis morbidity trends in Russia in 2009-2013. Social aspects of population health. 2015, 41(1). 7-15. (rus)

*
Znamenskaya L.F., Melekhina L.Ye., Bogdanova Ye.V., Mineyeva A.A. Psoriasis incidence and prevalence in the Russian Federation. Vestnik dermatologii i venerologii. 2012 (5), 20-29. (rus)

Michalek IM, Loring B, John SM. A systematic review of worldwide epidemiology of psoriasis. J Eur Acad Dermatol Venereol. 2017 Feb;31(2):205-212. 27573025., # - only for adults (18 years and older).

Incidence statistics in Russian Federation. Estimated number of psoriatic patients (PP) in world.

Region	Population	PP
% of PD population in Russian Federation and other countries of former USSR (top assessment)		4%
Moscow and Moscow Region	17 000 000	680 000
Other regions of Russia	125 000 000	5 000 000
Countries of former USSR (except	150 000 000	6 000 000
% of PD population in world (on average)		2%
Population of all countries of world	7 300 000 000	146 000 000

PD - psoriatic disease

Basic researches

Many of PP had malabsorption syndrome. Zhanna Rudkovskaya, etc. (2003),
PRNRMU, Institute of food of Russian Academy of Medical Science, Moscow, Russia.
Eugeny Kharkov with co-workers (from 2005 till now). Krasnoyarsk state medicine university, Krasnoyarsk, Russia.

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Majority of PP had SIBO (small intestine bacterial overgrowth).
More than 10^5 CFU/ml found in 95 of 121 PP (78,5%).
Natalia Potaturkina-Nesterova with co-workers (2007-9). Ulyanovsk State University, Ulyanovsk, Russia.

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Majority of PP had high blood LPS-level.
Zuhra Garaeva with co-workers (2005-7). Kazan Medicine Academy, Kazan, Russia.

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Phagocyte tolerization (reprogramming) and their properties.
Robert Sabat and Kerstin Wolk with co-workers (2000-2005). University Hospital Charité, Berlin, Germany.
Jean-Marc Cavaillon with co-workers (from 2004 till now). Institut Pasteur, Paris, France.

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Systemic model of pathogenesis (BF-model). The antigenic role of streptoccocal peptidoglycan outside skin (gut, tonsils, blood flow) and inside psoriatic skin.
Barbara Baker and Lionel Fry (2006-7). Faculty of Medicine, Imperial College, London, UK.

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New systemic model of pathogenesis (Y-model). Development of BF-model. Detailed coordinated description of systemic and local subprocesses. Mikhail Peslyak (2012). Moscow, Russia (2012).

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Netosis of neutrophils in psoriatic patients' blood and skin. Demonstration of the correlation between netotic neutrophil percentage in blood and psoriasis severity. Correlation between netotic neutrophil percentage in control blood of healthy patients under the influence of serum from psoriatic patients and severity of their PASI. Estimation of netotic neutrophil quantity in psoriatic skin. Cheng-Che E. Lan et al., Department of Dermatology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan. (Hu 2016).

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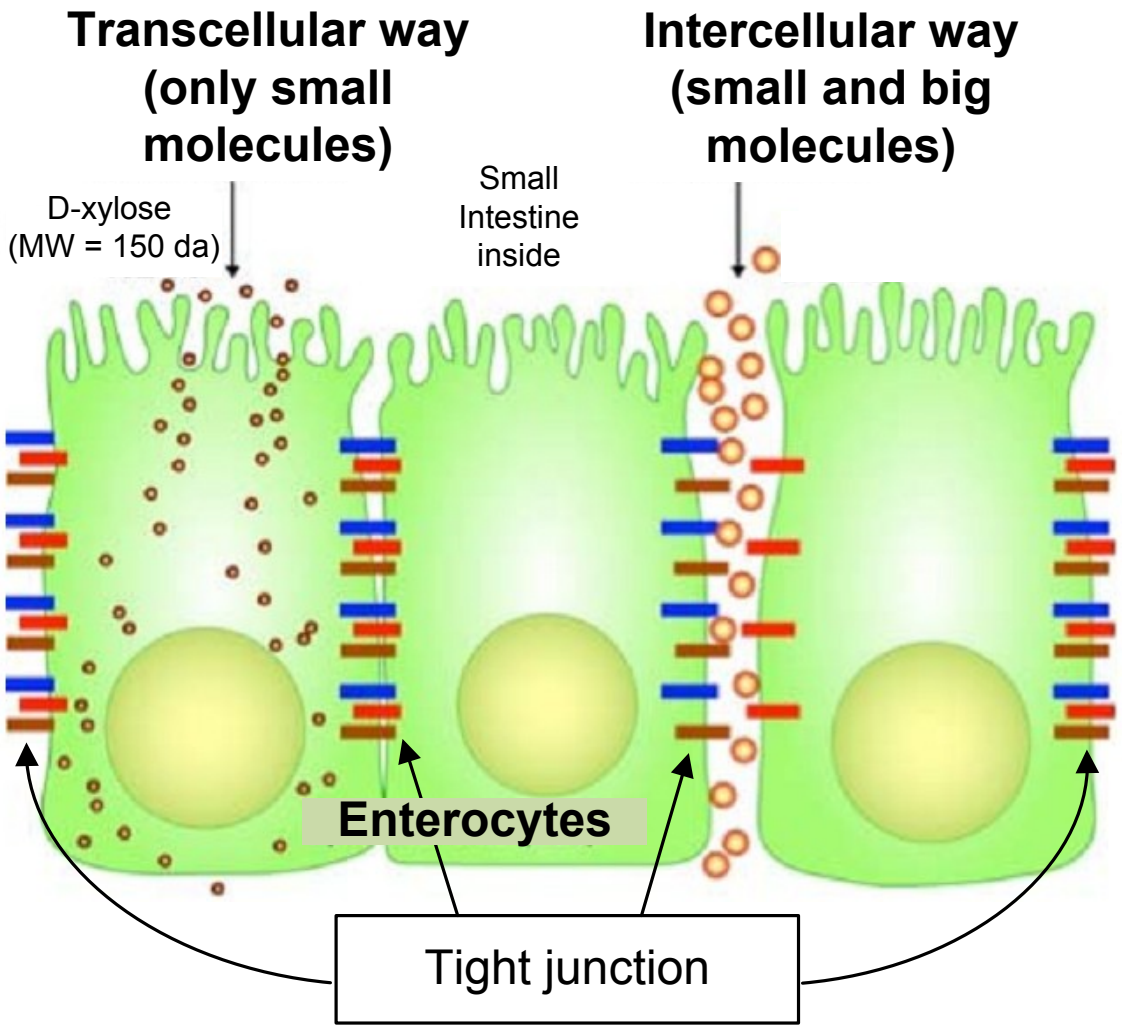
Definition of whole blood metagenome. Long-term studies at various diseases. Whole blood metagenome in healthy people was first identified (by 16S-test). Remy Burcelin et al., Vaiomer, LABEGE, France, Benjamin Lelouvier et al., Institute of Cardiovascular and Metabolic Diseases, Toulouse, France (2011-2016). (Paisse 2016)

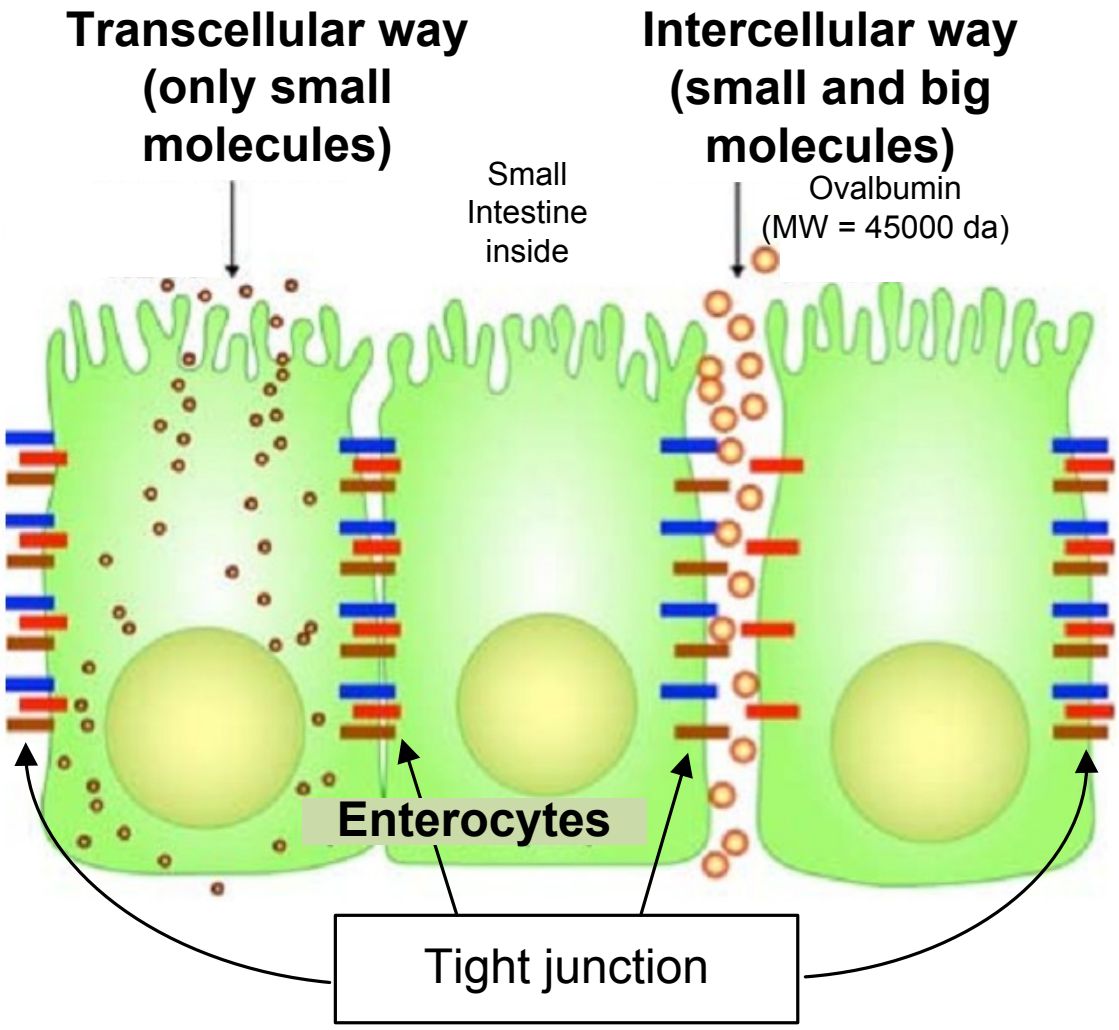
Transcellular small intestine permeability at psoriasis. D-xylose test.



The subject of works was also relations between malabsorption syndrome (SM) and psoriasis (Harkov 2008, Harkov 2006, Harkov 2005). SM grade can be measured in grams of D-xylose excreted with urine within 5 hours after oral taking. SM was diagnosed in 83 psoriatics and 20 persons of control group. It was rather lower in psoriatics (average value SM=1.0) in comparison with standard (average value SM=1.8). They found inverse relationship between SM and severity (PASI) and type of psoriasis: vulgar (SM=1.2, PASI=14), exudative or arthropathic (SM=1.0; PASI=18), erythrodermic (SM=0.8; PASI=39). Also they found that the lower SM, the longer is disease duration. These results are represented in the dissertation (Shiryaeva 2007) in more details and with a larger group of psoriatics (103 patients).

Harkov EI, Prohorenkov VI, Shiryaeva YuA. Indices of functional activity of small intestine in patients with psoriasis. Siberian Medical Review, 2008;(6):55-58. (Rus).
Harkov EI, Shiryaeva YuA, Teryoshina DS. Malabsoption syndrome and psoriasis: the method of correction. Siberian Medical Journal (Irkutsk), 2006;(7):61-63. (Rus).
Harkov EI, Shiryaeva YuA. Malabsorption syndrome in psoriasis: clinical-laboratory parallels. Siberian Medical Review, 2005;(2-3):62-64. (Rus).
Shiryaeva YuA. Malabsorption syndrome at psoriatics. Dissertation, Krasnoyarsk, 2007, 150 p. (Rus),
Vojdani A. For the assessment of intestinal permeability, size matters. Altern Ther Health Med. 2013 Jan-Feb;19(1):12-24. 23341423.

Intercellular small intestine permeability at psoriasis. Ovalbumin test.



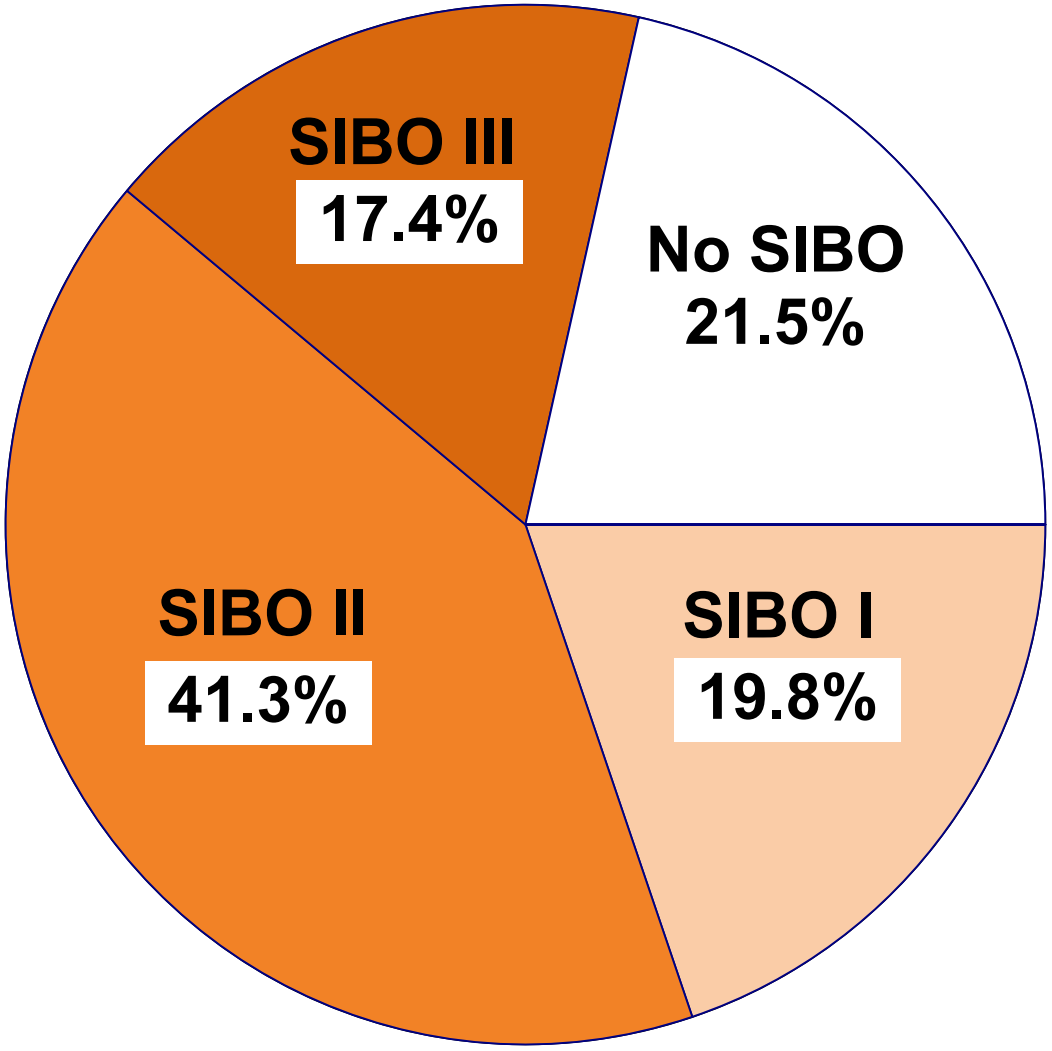
Ovalbumin (OVA) test was used to evaluate the level of intestinal permeability in children with psoriasis (Parfenov 1999, Rudkovskaya 2003, Stenina 2004). Standard blood OVA-level before OVA-load (chicken egg proteins) is close to zero and it shouldn't exceed 1 ng/ml after 3 hours of OVA-load. Initial average OVA-level of 30 children was 1.13 ng/ml and average OVA-level after OVA-load was 15.5 ng/ml (maximum - 104 ng/ml). Average OVA-level in children with advanced psoriasis was 35.4 ng/ml, in children with stable psoriasis - 5.1 ng/ml.

OVA-permeability depends on disease duration in children with advanced psoriasis. It sharply increases for first four months and then doesn't essentially vary. OVA-permeability decreased from 43.2 ng/ml to 23.1 ng/ml during treatment in patients with subacute psoriasis. There was no obvious correlation between OVA-permeability and psoriasis severity (index PASI).

Stenina MA, Kulagin VI, Rudkovskaya ZV et al, Role of disturbances of intestine barrier function in pathogenesis of psoriasis in children, Russian Journal of Skin and Sexually Transmitted Diseases, 2003;(2):20-23. (Rus), ISSN 1560-9588.
Rudkovskaya ZV, Clinical and laboratory monitoring of efficiency of application of method of interval normobaric hypoxia in complex treatment of psoriasis in children. Dissertation, Moscow, 2003, 137 p. (Rus).
Vojdani A. For the assessment of intestinal permeability, size matters. Altern Ther Health Med. 2013 Jan-Feb;19(1):12-24. 23341423.

SIBO (small intestinal bacterial overgrowth) at psoriasis

Level SIBO (small intestinal bacterial overgrowth) more than 10^5 CFU/ml (TBC > 5) was found at 95 psoriatic patients (78.5%).



No SIBO
TBC less than 10^5 CFU/ml.

78.5%

SIBO I. No anaerobic. Normal aerobic TBC from 10^5 to 10^6 CFU/ml.

SIBO II. Occurrence of anaerobic. TBC from 10^6 to 10^7 CFU/ml.

SIBO III. Prevalence of anaerobic. TBC more than 10^7 CFU/ml

TBC is total bacterial count.
CFU is colony-forming unit.

SIBO. Transient microflora of proximal small intestine.

Microflora	Psoriatic patients (121 pers.) carrier	% of carrier	lg CFU/ml	Control healthy (43 pers.) carrier	% of carrier	lg CFU/ml
Bifidobacterium spp.	112	93%	5.3	17	40%	2.41
Lactobacillus spp.	102	84%	4.66	8	19%	2.54
Bacteroides spp.	20	17%	3.3	5	12%	2.86
E.coli typical	81	67%	5.04	11	26%	2.94
E.coli lactose-neg.	4	3%	3.62	0		
E.coli hemolytic	18	15%	3.6	0		
Enterococcus spp.*	79	65%	5.28	0		
Str.viridans	36	30%	5.74	0		
S.aureus	18	15%	3.24	0		
Str.pyogenes	11	9%	4.81	0		
S.epidermidis	75	62%	5.54	17	40%	2.70
Candida	45	37%	4.76	10	23%	2.43
Acinetobacter spp.	7	6%	3.56	4	9%	2.40
Proteus spp.	24	20%	4.1	7	16%	2.14
Clostridium spp.	24	20%	5.2	0		
Klebsiella spp.	17	14%	3.13	0		
Moraxella spp.	63	52%	4.45	0		
Total bacterial count			**6.49**			**3.05**

Most of PP have SIBO.
N. I. Potaturkina-Nesterova with coauthors (2009-11). Ulyanovsk State University.

It is presented at world conference on treatment of psoriasis and psoriatic arthritis in 2012.

Report on Internet.

* - was defined to within look only for part of patients, in 90% it was E.faecalis.

	Species / Patient	1	2	3	7	8	9	10	11	12	14	15	18	20	22	23	25	26	27	39	40
	Candida albicans	3	3	3	3		4			<3	2	3	2		2				4		
	Candida lusitaniae								2												
	Bifidobacterium sp.									7					3						
	Enterococcus avium							4													
	Enterococcus casseliflavus																			4	
	Enterococcus durans/hirae							3													
	Enterococcus faecalis	5	2	3						3			2			3			3		5
	Escherichia coli (лактозонегативная)												3							4	6
	Gemella haemolysans								<3												
	Klebsiella pneumonia																			4	6
	Kocuria kristinae											3									
	Lactobacillus sp.						4								3						
	Staph.aures		3		3					3		4								3	4
	Staph.auricularis										5										
	Staph.epidermidis													3	3			4			
	Staph.lugdunensis				3																
	Staph.saprophyticus	4											2			2					
	Stenotrophomonas maltophilia					<3															
	Strep.agalactiae		3							4											
	Strep. anginosus (milleri subgroup)							<=4												4	4
	Strep.dysgalactiae											4									
	Strep.equinus								3												
	Strep.infantarius, subsp.infantarius				4																
	Strep.mitis/oralis	5	3	6	<3				4	<3	6		3	4	4		3	5	5		
	Strep.mutans															3					
	Strep.pneumoniae							3													
	Strep.salivarius																	5			
	Peptostr.anaerobis	4																			
	Pseudomonas alcaligenes										5										
	SIBO level	**5**	**3**	**6**	**4**	**<3**	**4**	**4**	**4**	**7**	**6**	**4**	**3**	**4**	**4**	**3**	**3**	**5**	**5**	**4**	**6**

10

SIBO_Moscow e2.2

Small intestine microbiome of PP in Treitz ligament, lg (CFU/ml).

Researches are executed in "National Medical and Surgical Center named after N.I. Pirogov".

These species are presumed psoriagenic.

Some species (or strains) are presumed psoriagenic.

Symbols

PG	PG – any peptidoglycan (in particular PG-Y)
Y-antigen	Y-antigen = part(s) of interpeptide bridge IB-Y
PG-Y	PG-Y = peptidoglycan with interpeptide bridges IB-Y
PsB	PsB = psoriagenic bacteria = Gram+ bacteria with peptidoglycan PG-Y.
LPS	LPS = lipopolysaccharide, free and bound in complexes
	Gram(-) TLR4-active bacteria
	Enterocytes - epithelial cells covering mucous intestine
	EC - endothelial cells
KC	KC - keratinocytes

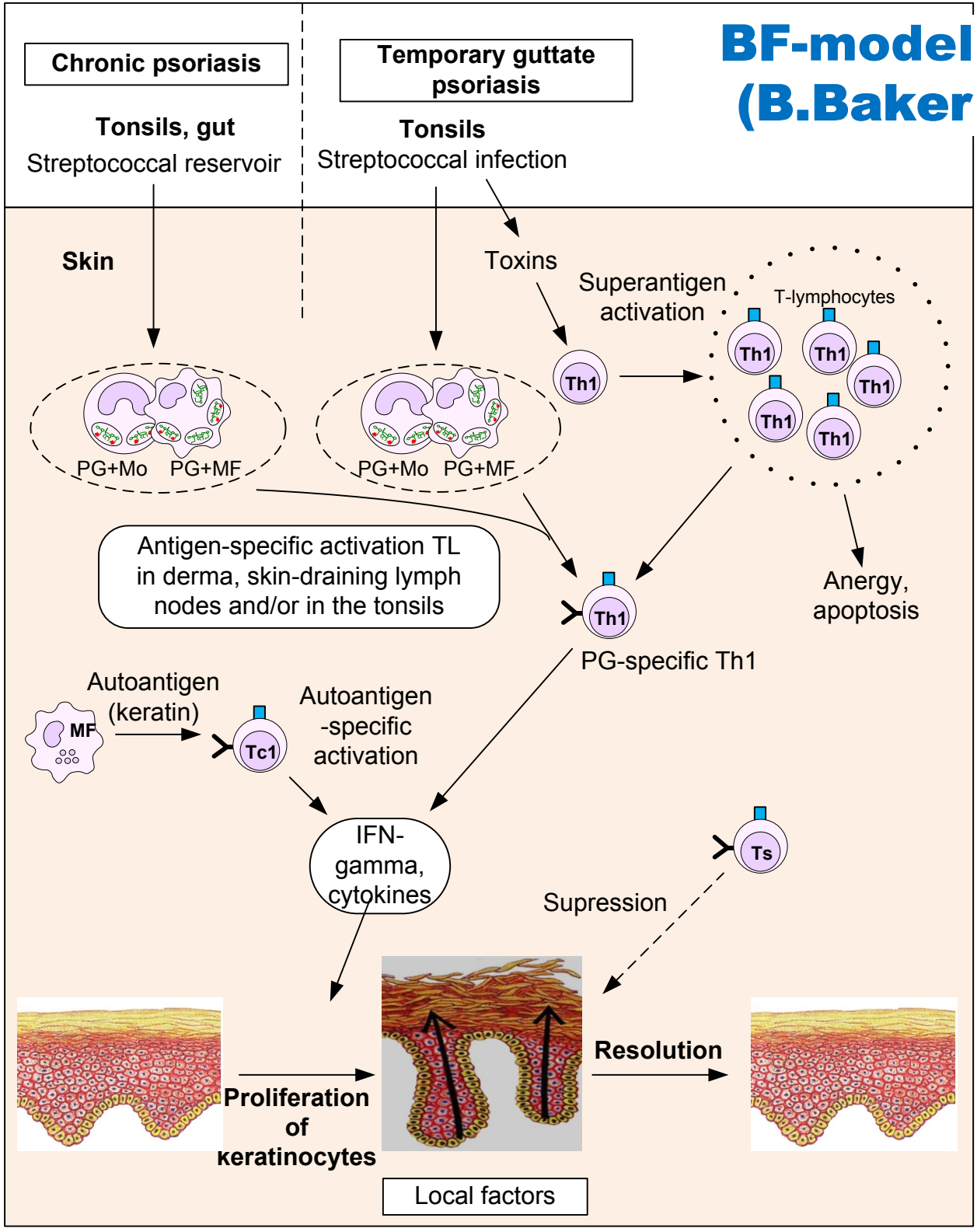
	Gram+ and Gram(-) bacteria - intestine commensals
	nhDNA – non-human DNA (in particular bacDNA)
	nhDNA resident origin in psoriatic skin
	nhDNA non-resident origin in psoriatic skin
TL	T-lymphocytes
TL-Y	Y-specific T-lymphocytes
Neu	Neu - neutrophils
Neu-Y	Neu-Y = PG-Y(+)Neu
NET	NET – netotic products from Neu and Neu-Y

Mo	Mo - monocytes
DC	DC – dendritic cells
MoDP	MoDP – resident stem cells - precursors of MF and MoDC in skin
MoDC	DC derived from Mo or from MoDP
MF	Macrophages derived from Mo or from MoDP
Mo-Y	Mo-Y = PG-Y(+)Mo
DC-Y	DC-Y = PG-Y(+)DC
MoDC-Y	MoDC-Y = PG-Y(+)MoDC
maDC-Y	maDC-Y = mature dendritic cells, presenting Y-antigen

Mo-T	Mo-T = tolerized monocytes
Mo-R	Mo-R = PG-Y(+)Mo-T
DC-T	DC-T - tolerized DC
DC-R	DC-R = PG-Y(+)DC-T
MoDC-T	DC derived from Mo-T
MoDC-R	DC derived from Mo-R
MF-T	Macrophages derived from Mo-T
MF-R	Macrophages derived from Mo-R
PDC	PDC - plasmacytoid dendritic cells

Used in Y-model only

BF-model of pathogenesis (B.Baker & L.Fry, 2006-7).



Process of initialization of temporary guttate psoriasis is simulated on right part of figure. Streptococci temporary situated in tonsils produce toxins-superantigens. They activate TL of tonsils or skin lymph nodes. PG-specific TL are selected due to contacts with PG+Mo (transformed to PG+MoDC). Other TL become anergy or apoptotic.
Similar sequence of events can be observed in chronic psoriasis (left part of figure) if streptococci and/or streptococcal antigens stay in tonsils and/or intestine for a long time. Plaques appear after PG+Mo and PG-specific TL enter in derma. Autoantigen (e.g. keratin) has aggravating effect.

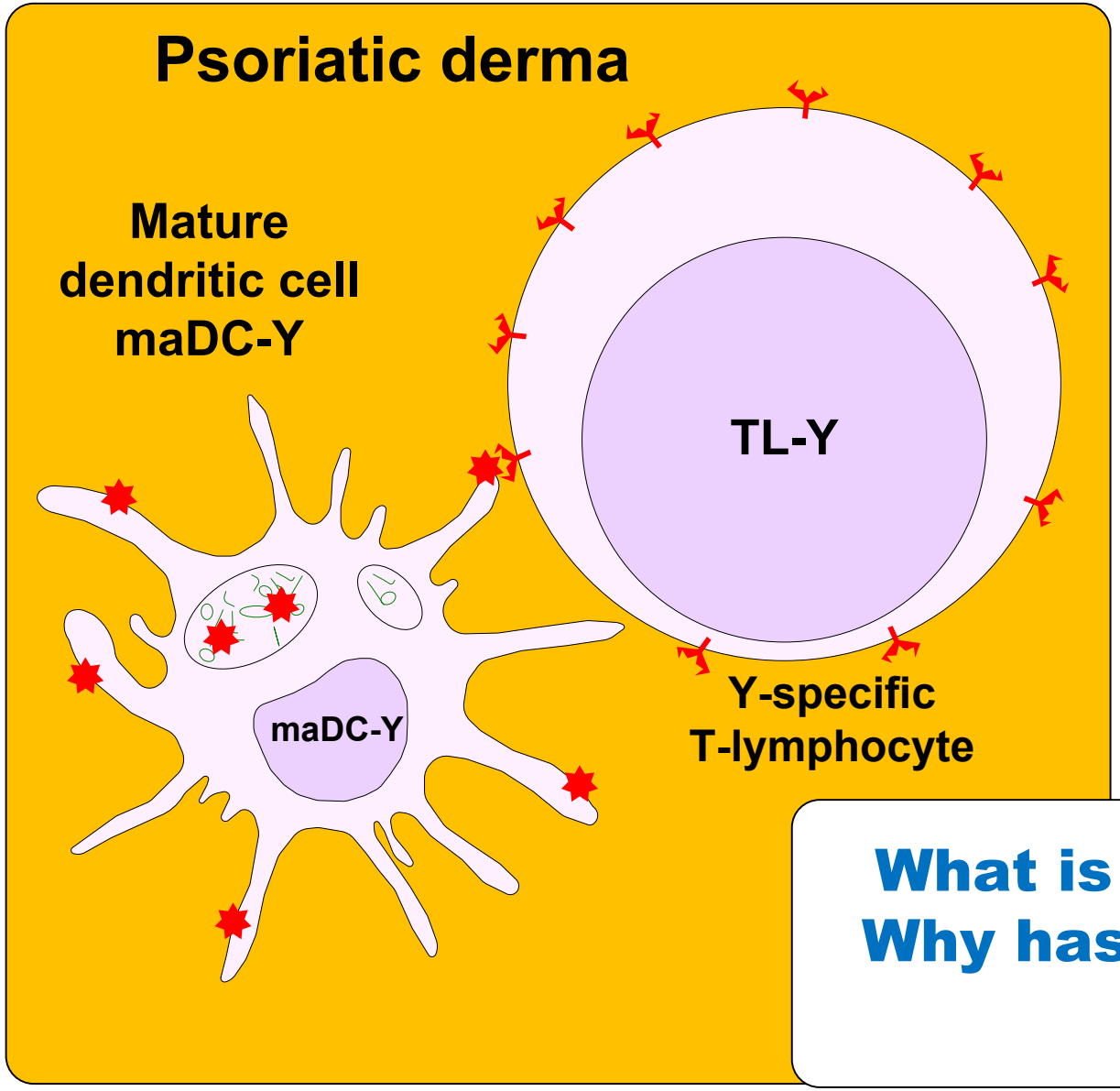
BF-model doesn't give answers to the next two questions:

1. Why do PG+Mo appear in skin though PG was endocytosed by Mo in other place of organism?

2. Why do PG+Mo become PG+MoDC and present PG?

Baker BS, Powles A, Fry L. Peptidoglycan: a major aetiological factor for psoriasis? Trends Immunol. 2006 Dec;27(12):545-51. 17045843.

Mature dendritic cell present unknown Y-antigen to T-lymphocyte



Key event of adaptive immune response, continually taking place in each psoriatic plaque.

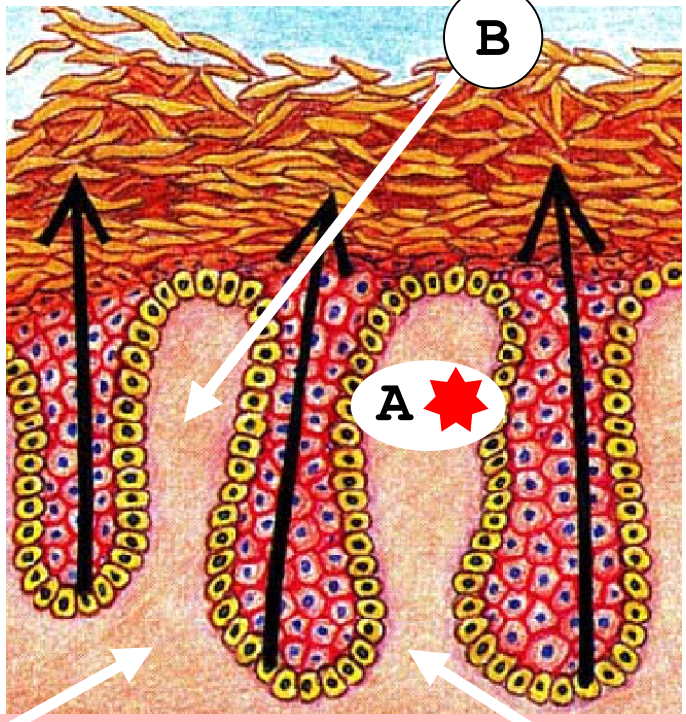
Y-antigen = unknown antigen

What is Why has chemical structure? appeared in psoriatic derma?

Versions of origin of unknown antigen

	Non-Host	Host
Resident	-	A
Non-resident from external environment	B	-
Non-resident from within (for example from blood flow)	C	D

Version B. Numerous researches have shown its insolvency.



Version C - the main version from authors of systemic models of pathogenesis. The known facts do not contradict it. It will be checked within this project.

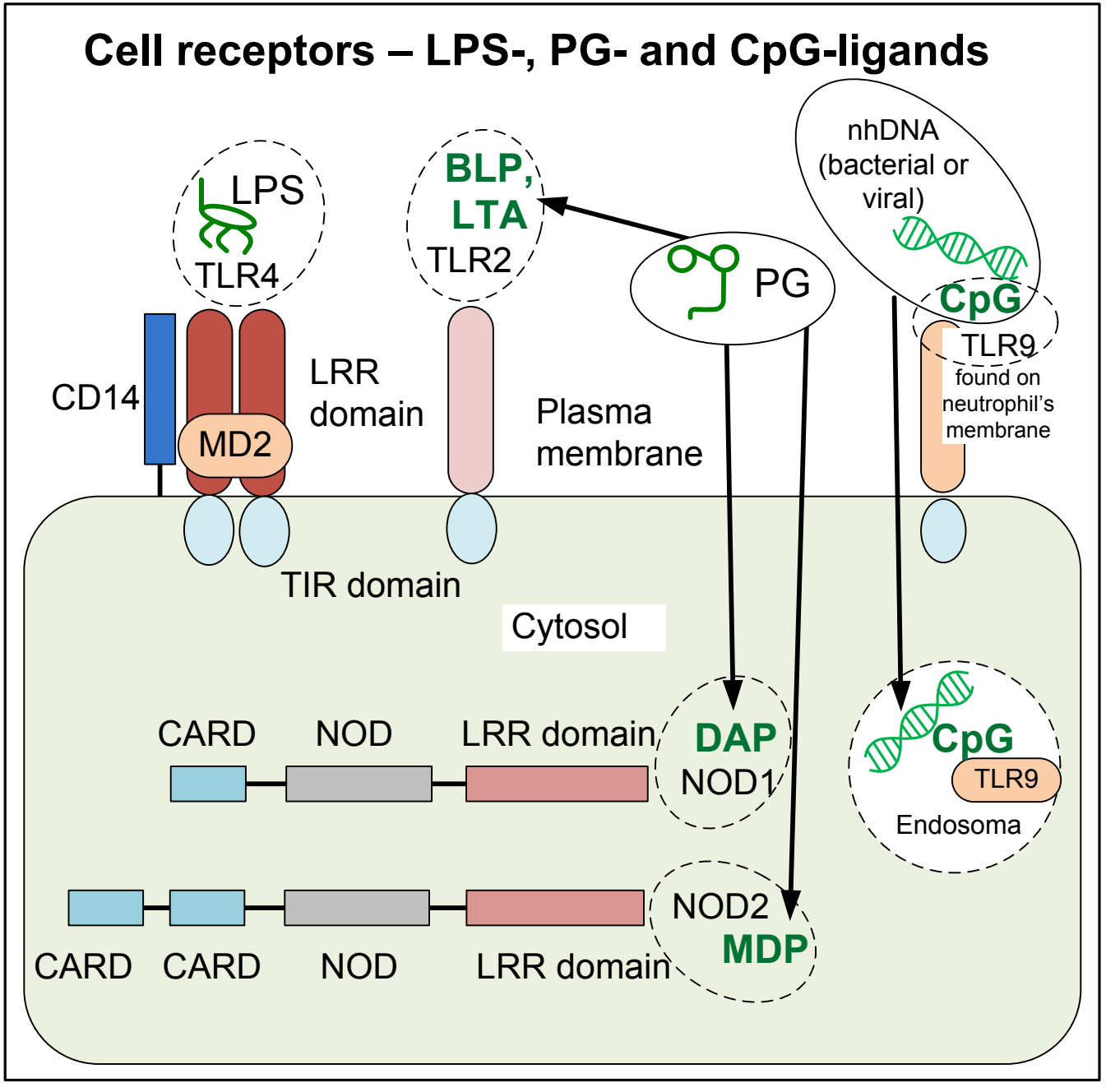
Version A -
The main version from authors of local models of pathogenesis. Numerous attempts to prove its solvency have not resulted in success yet.

Version D - antigen has host origin, but is not resident. This version is not proved (for the same reasons, as version A).

PAMP, structure and localization of TLR2, TLR4, TLR9, NOD1 and NOD2

PAMP (Pathogen-associated molecular patterns) - in particular
LPS - lipopolysaccharide (Gram (-) bacteria),
LTA lipoteichoic acids (Gram + bacteria),
PG - peptidoglycan (Gram + and Gram-of bacterium),
CpG (fragment of bacterial or virus DNA), etc.

TLR9 – CpG ligand;
TLR4 – LPS ligand;
Receptors of fragments of PG:
TLR2 – BLP ligand bacterial lipoprotein) and LTA;
NOD1 – DAP ligand (diaminopimelic acid);
NOD2 – MDP ligand (muramit dipeptide).
Activity of interaction of PAMP decides on its ligand by PAMP modification.



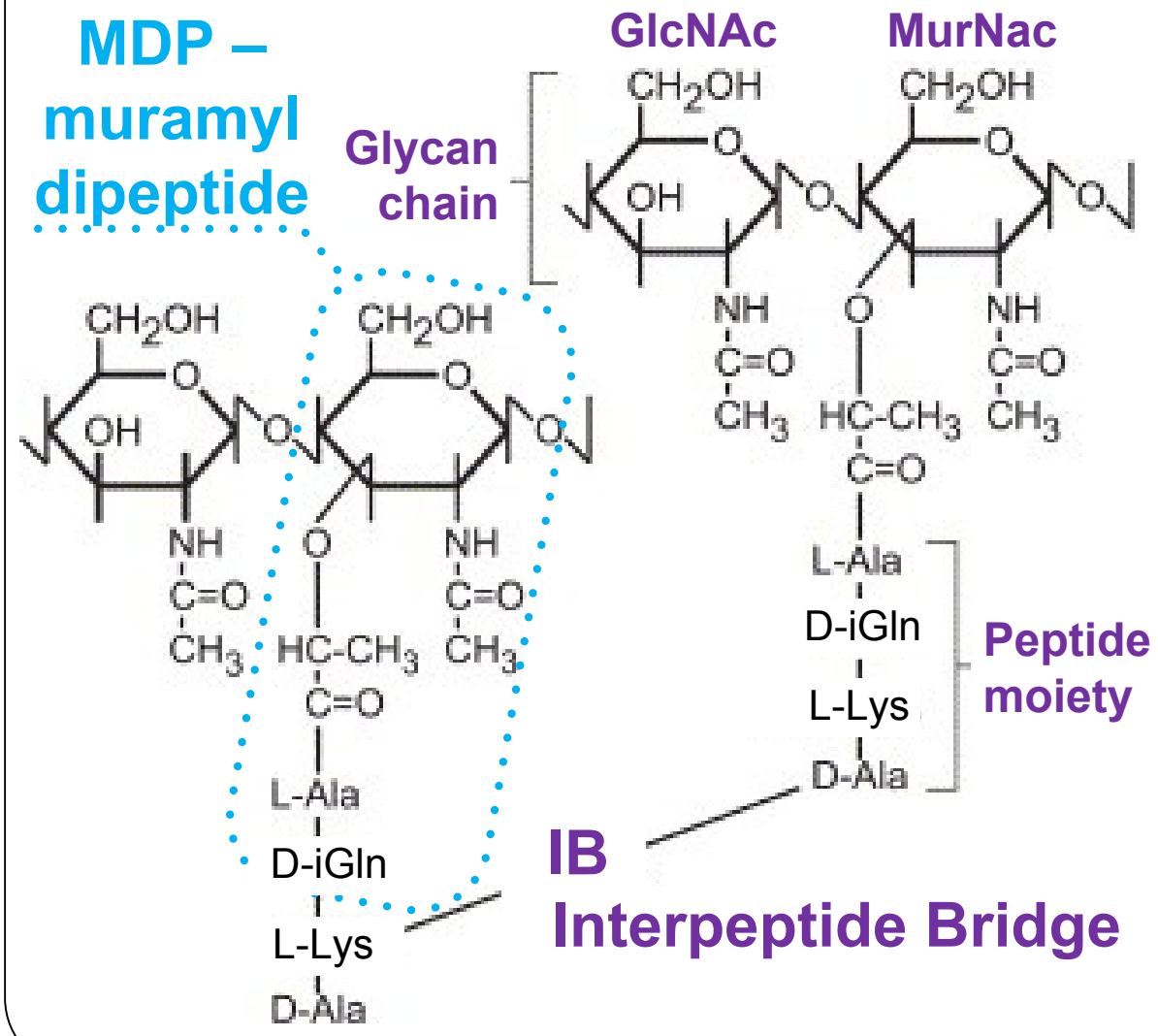
Peptidoglycan (PG) structure and PsB

PG

Peptidoglycan (any)

MDP – muramyl dipeptide

Glycan chain



Peptide moiety

IB Interpeptide Bridge

PG-Y

PG-Y – peptidoglycan with IB-Y

PsB

PsB - bacteria presumed psoriagenic	Interpeptide Bridge	Sourses
Str.pyogenes	(L-Ala)(2-3) or (L-Ser)-(L-Ala)	#, KEGG
Almost all from Streptococcus sp.	(L-Ala)(1-3) or (L-Ser)-(L-Ala)	#, KEGG
Enterococcus faecalis	(L-Ala)(2-3)	#, KEGG
Many from Leuconostoc sp.	(L-Ala)(2) or (L-Ala)-(L-Ser) or (L-Ser)-(L-Ala)(1-2)	#, KEGG
Many from Weissella sp.		
Some from Bifidobacterium sp.	(L-Ala)(2-3) or (L-Ser)-(L-Ala)	#

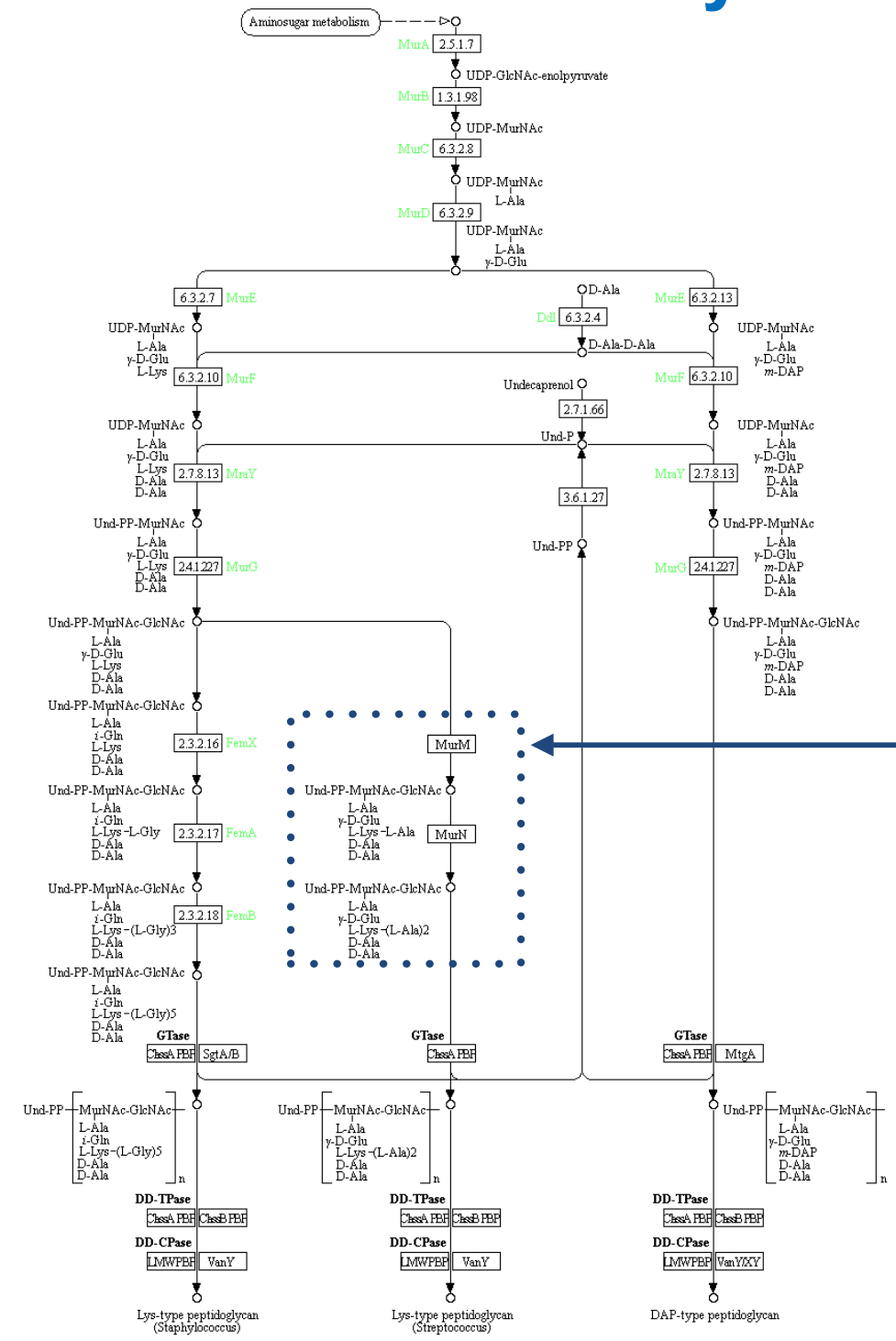
- scientific works

KEGG - Kyoto Encyclopedia of Genes and Genomes

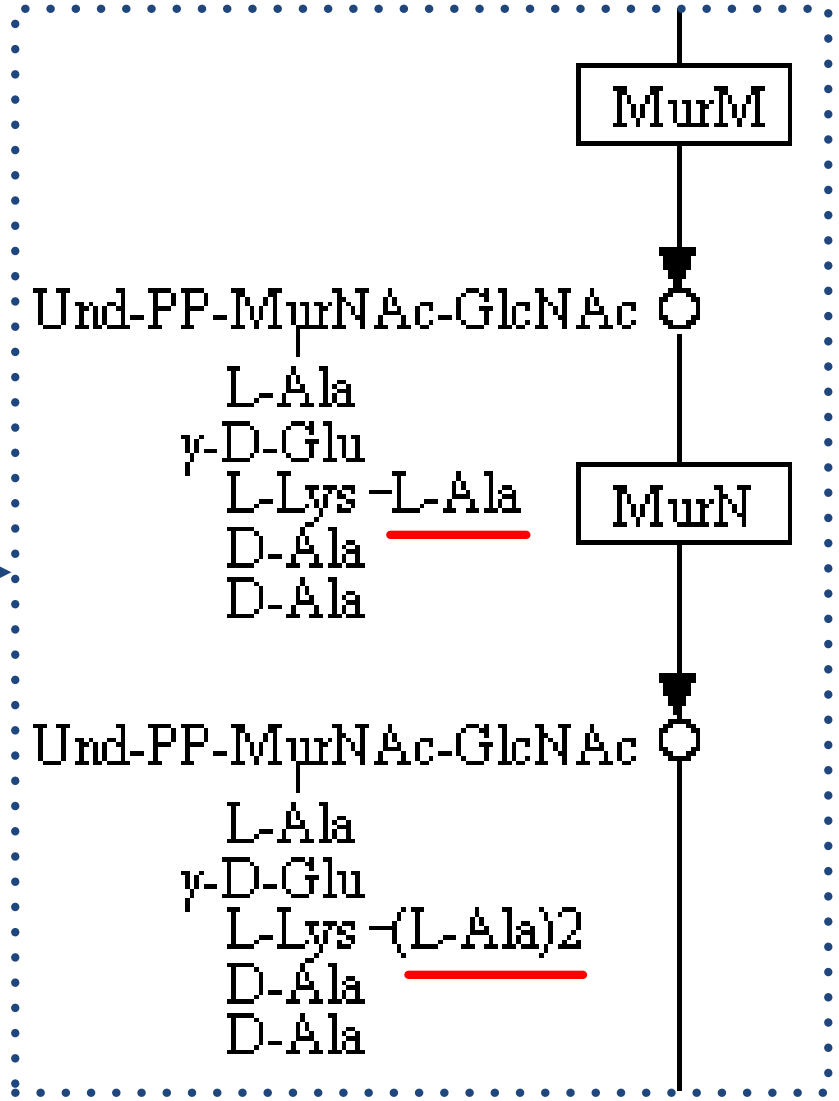
?

Y-antigen = part(s) of interpeptide bridge **IB-Y**

Biosynthesis of peptidoglycan



Biosynthesis of interpeptide bridge with participation of enzymes like murM and murN

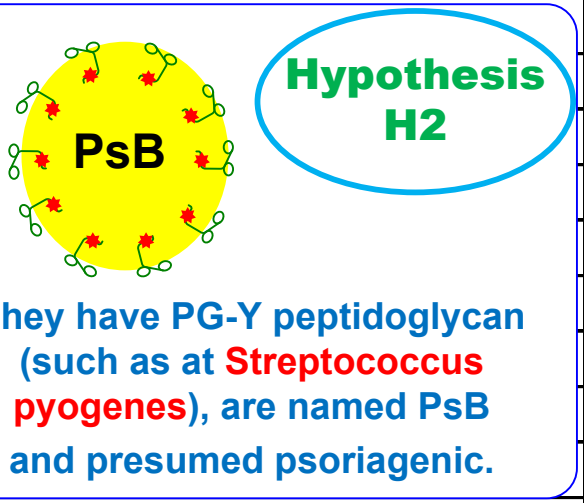


www.kegg.jp

00550 4/10/17
(c) Kanehisa Laboratories

Species of Gram+ bacteria with interpeptide bridges IB-Y. IB-Y = (L-Ala)-(L-Ala) or (L-Ser)-(L-Ala). (KEGG database).

Streptococcus sp.		Species from other genus
Streptococcus agalactiae	Streptococcus pseudopneumoniae	Enterococcus faecalis
Streptococcus anginosus	Streptococcus pyogenes	Enterococcus silesiacus
Streptococcus constellatus	Streptococcus salivarius	Eubacterium sulci
Streptococcus cristatus	Streptococcus sanguinis	Lactococcus garvieae
Streptococcus dysgalactiae	Streptococcus suis	Lactococcus piscium
Streptococcus equi	Streptococcus thermophilus	Lactococcus raffinolactis
Streptococcus gallolyticus	Streptococcus uberis	Leuconostoc carnosum
Streptococcus gordonii	Streptococcus vestibularis	Leuconostoc citreum
Streptococcus infantarius		Leuconostoc garlicum
Streptococcus iniae		Leuconostoc gelidum
Streptococcus intermedius		Leuconostoc kimchii
Streptococcus lutetiensis		Leuconostoc lactis
Streptococcus macedonicus		Leuconostoc mesenteroides
Streptococcus mitis		Melissococcus plutonius
Streptococcus mutans		Oenococcus oeni
Streptococcus pantholopis		Weissella ceti
Streptococcus parasanguinis		Weissella cibaria
Streptococcus parauberis		Weissella jogaejeotgali
Streptococcus pasteurianus		Weissella koreensis
Streptococcus pneumoniae		Weissella paramesenteroides



Almost all strains of these species have peptidoglycan similar to Str.pyogenes peptidoglycan. Therefore these species are presumed psoriagenic. Formation of interpeptide bridges is facilitated by various murMN-genes.
KEGG database makes it possible to determine all (included in it) strains of bacteria which have genes ensuring secretion of both enzymes, i.e. murM and murN types. DB KEGG is being updated.

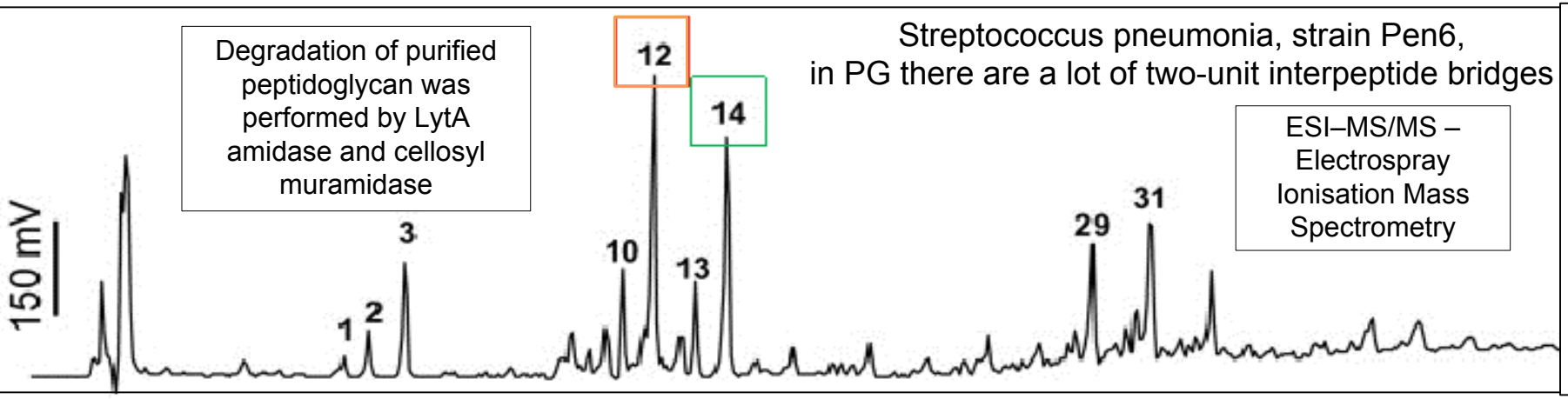
species 2018

Muropeptides and peptides which are formed at degradation of Str.pneumonia peptidoglycan.

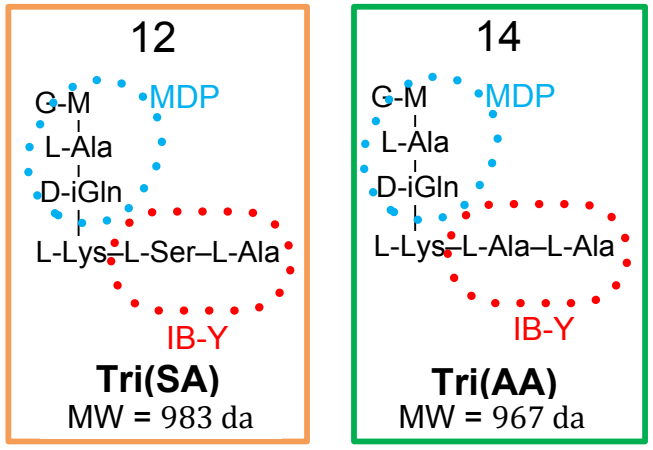
Degradation of purified peptidoglycan was performed by LytA amidase and cellosyl muramidase

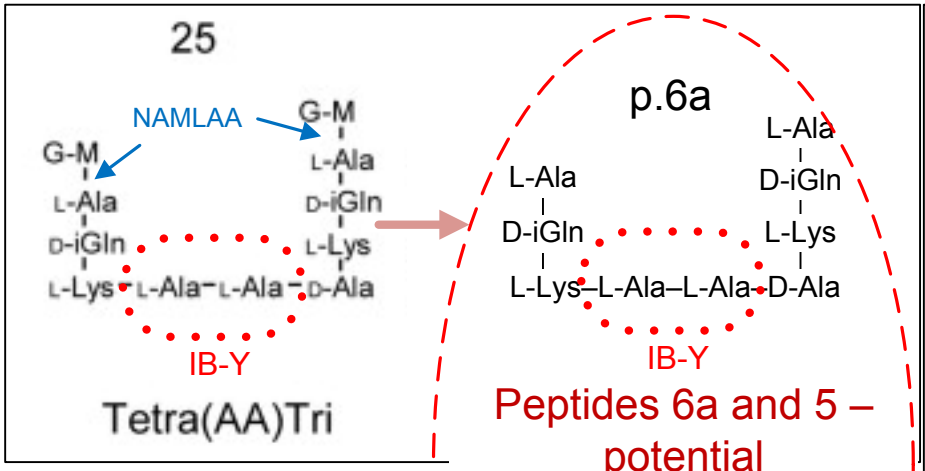
Streptococcus pneumonia, strain Pen6, in PG there are a lot of two-unit interpeptide bridges

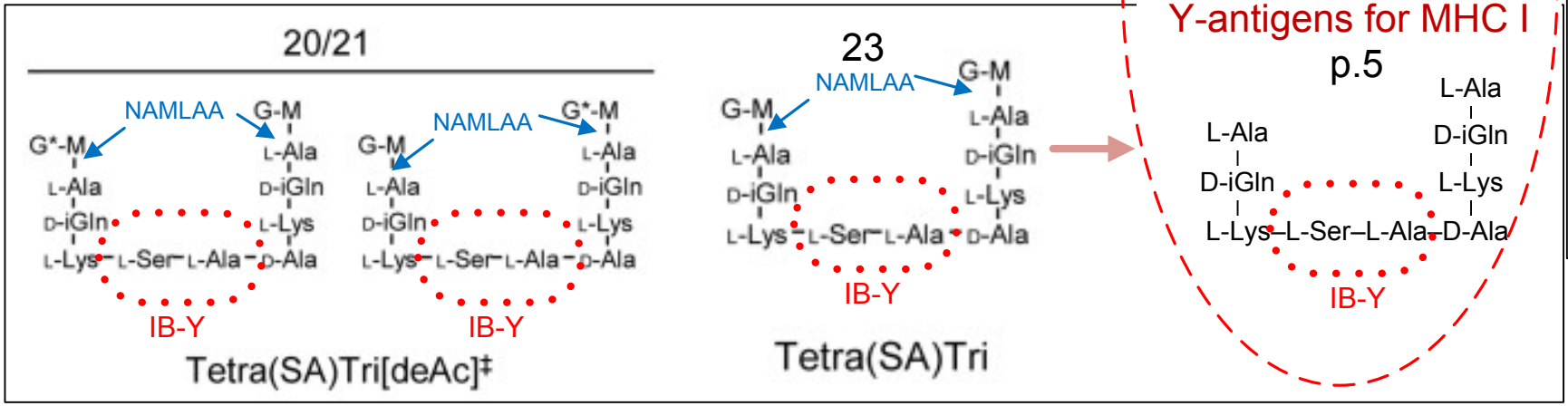
ESI–MS/MS – Electrospray Ionisation Mass Spectrometry



Muropeptides 12 and 14 constitute an essential part of PG fragments (more than 20%) of Pen6 strain, and include MDP (NOD2 ligand) and IB-Y.
Their proportion in the total amount is determined by % of interpeptide bridges (L-Ser)-(L-Ala) and (L-Ala)-(L-Ala) in particular strain.







Structure of some muropeptides (37 in total were found).

Muropeptides 20, 21, 23 and 25 after being affected by intracellular amidase NAMLAA (= PGRP2) form linear peptides 5 and 6a, respectively.

Linear peptides 5 and 6a contain epitope IB-Y and have 9 amino acids' length, which allows them to be presented through MHC I. MDP, being adjuvant, ensures intensity of processing and presentation.

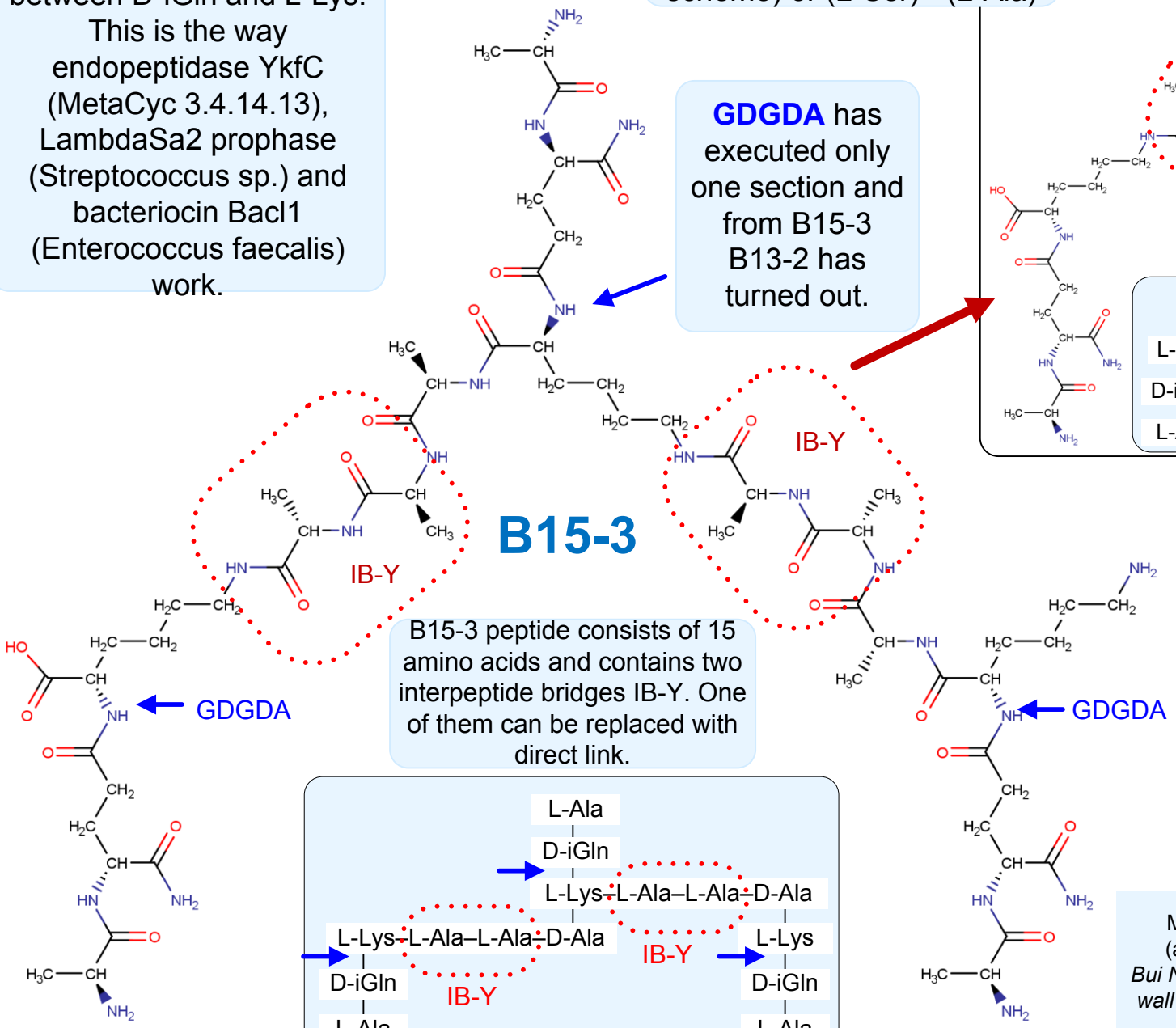
Bui NK, Eberhardt A, Vollmer D. et al. Isolation and analysis of cell wall components from Streptococcus pneumoniae.Anal Biochem. 2012 Feb 15;421(2):657-66. 22192687.

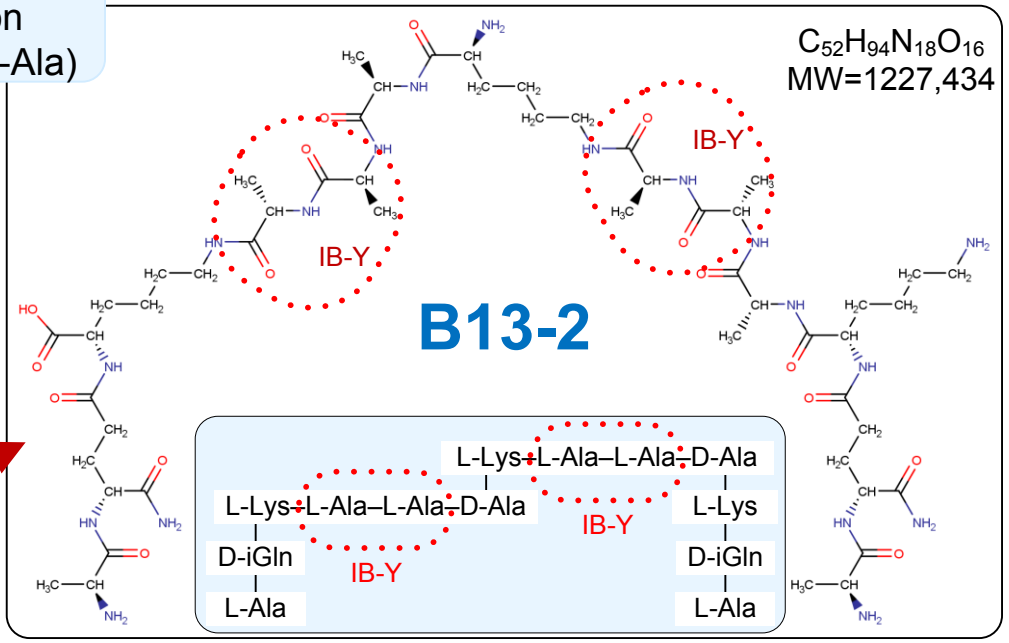
B13-2 peptide - potential Y-antigen.

GDGDA (conventional term) is endopeptidase which cuts the connection between D-iGln and L-Lys. This is the way endopeptidase YkfC (MetaCyc 3.4.14.13), LambdaSa2 prophase (Streptococcus sp.) and bacteriocin Bacl1 (Enterococcus faecalis) work.

IB-Y = (L-Ala) - (L-Ala) (as in formula and on scheme) or (L-Ser) - (L-Ala)

GDGDA has executed only one section and from B15-3 B13-2 has turned out.





$C_{52}H_{94}N_{18}O_{16}$
MW=1227,434

B15-3 peptide consists of 15 amino acids and contains two interpeptide bridges IB-Y. One of them can be replaced with direct link.

Depending on number of cuts performed by GDGDA, B15-3 peptide produces peptides from 13 amino acids (three options - one of them being B13-2), from 11 amino acids (three options) or from 9 amino acids.

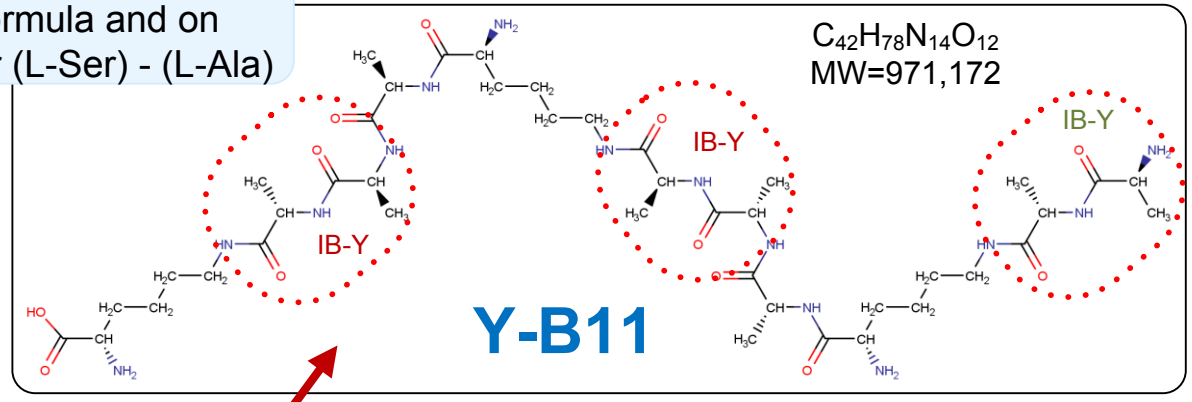
B15-3 peptide (and its analogs), as well as some of its derivatives (at least of 11 amino acids) are potential Y-antigens, presented through MHC II. In this case one or both IB-Y bridges act as epitope. The role of MDP as adjuvant is similar.

Muropeptides (33,34 and 35) - precursors of B15-3 peptide (at IB-Y = (L-Ser) - (L-Ala)) are for the first time classified in *Bui NK, Eberhardt A, Vollmer D. et al. Isolation and analysis of cell wall components from Streptococcus pneumoniae.Anal Biochem. 2012 Feb 15;421(2):657-66. 22192687.*

Y-B11 peptide - potential Y-antigen.

GDGDA (conditional name) - endopeptidase which cutting connection between D-iGln and L-Lys.
So work endopeptidase of YkfC (MetaCyc 3.4.14.13), LambdaSa2 prophase (Streptococcus sp.) and bacteriocin BacI1 (Enterococcus faecalis).

IB-Y = (L-Ala) - (L-Ala) (as in formula and on scheme) or (L-Ser) - (L-Ala)



GDGDA has executed all three sections.

Depending on the number of cuts, **peptide IX** produces peptides from 15 amino acids (three options), from 13 amino acids (three options) or linear **Y-B11 peptide** from 11 amino acids (the chart above).

Peptide IX contains three interpeptide bridges. One of the two central ones can be replaced with direct link.

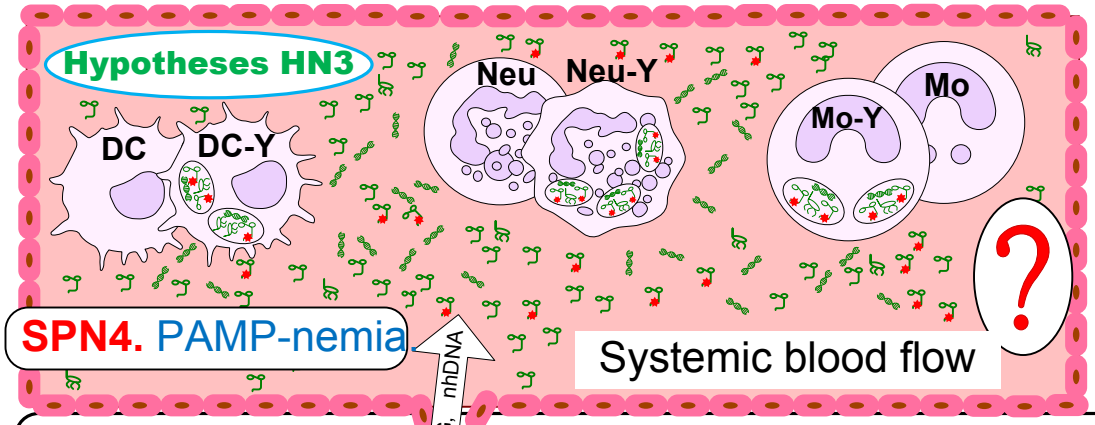
Peptide IX and any of the derived peptides can be the expected Y-antigen, presented through MHC II.
Only two central bridges can act as epitope.

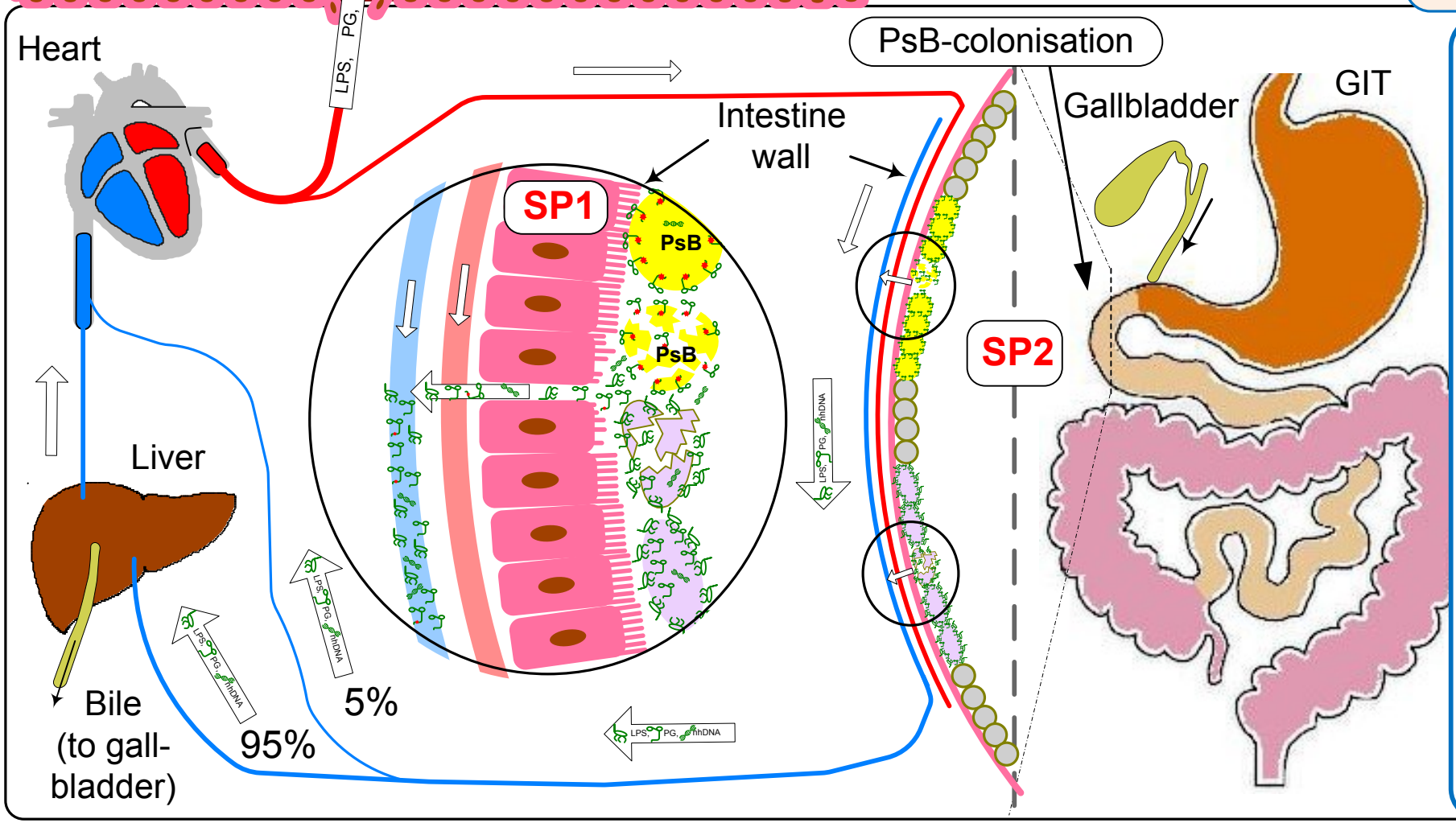
For the first time, **Peptide IX** was found in *Filipe SR, Severina E, Tomasz A. Distribution of the mosaic structured murM genes among natural populations of Streptococcus pneumoniae. J Bacteriol. 2000 Dec;182(23):6798-805. 11073926.*
In the same study, many similar and derived peptides are found. Their presence depends on Str.pneumoniae strain.

YN-model. Systemic psoriatic process SPPN and PAMP-nemia.

PAMP - Pathogen-associated molecular patterns
PAMP-nemia – chronic increasing of kPAMP-load (binding, endocytosis) on blood phagocytes, resulting in
- increased kPAMP concentration in blood;
- increased kPAMP-carriage of phagocytes.

kPAMP (key PAMP) are LPS (lipopolysaccharide), PG (peptidoglycan) and bacDNA (bacterial DNA).





Original causal subprocesses in small intestine: ?

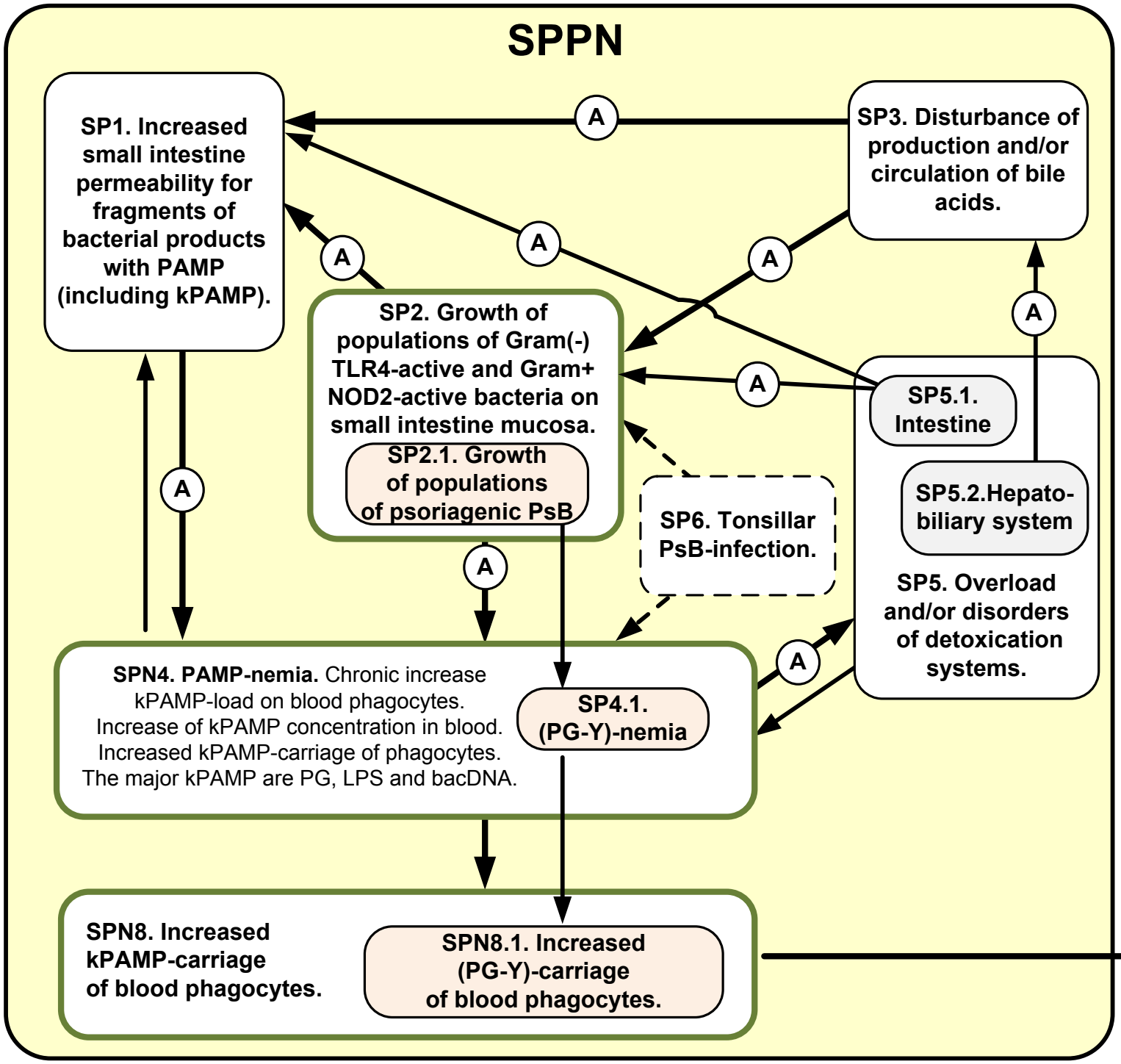
Hypotheses H1-1

SP1. Hyperpermeability for bacterial products.

Hypotheses H1-2

SP2. Growth of populations of Gram+ (including psoriagenic PsB) and Gram(-) TLR4-active bacteria.

YN-model. Systemic psoriatic process SPPN.



Interaction of subprocesses.

A **Vicious cycle links**

The main difference between SPPN and SPP.

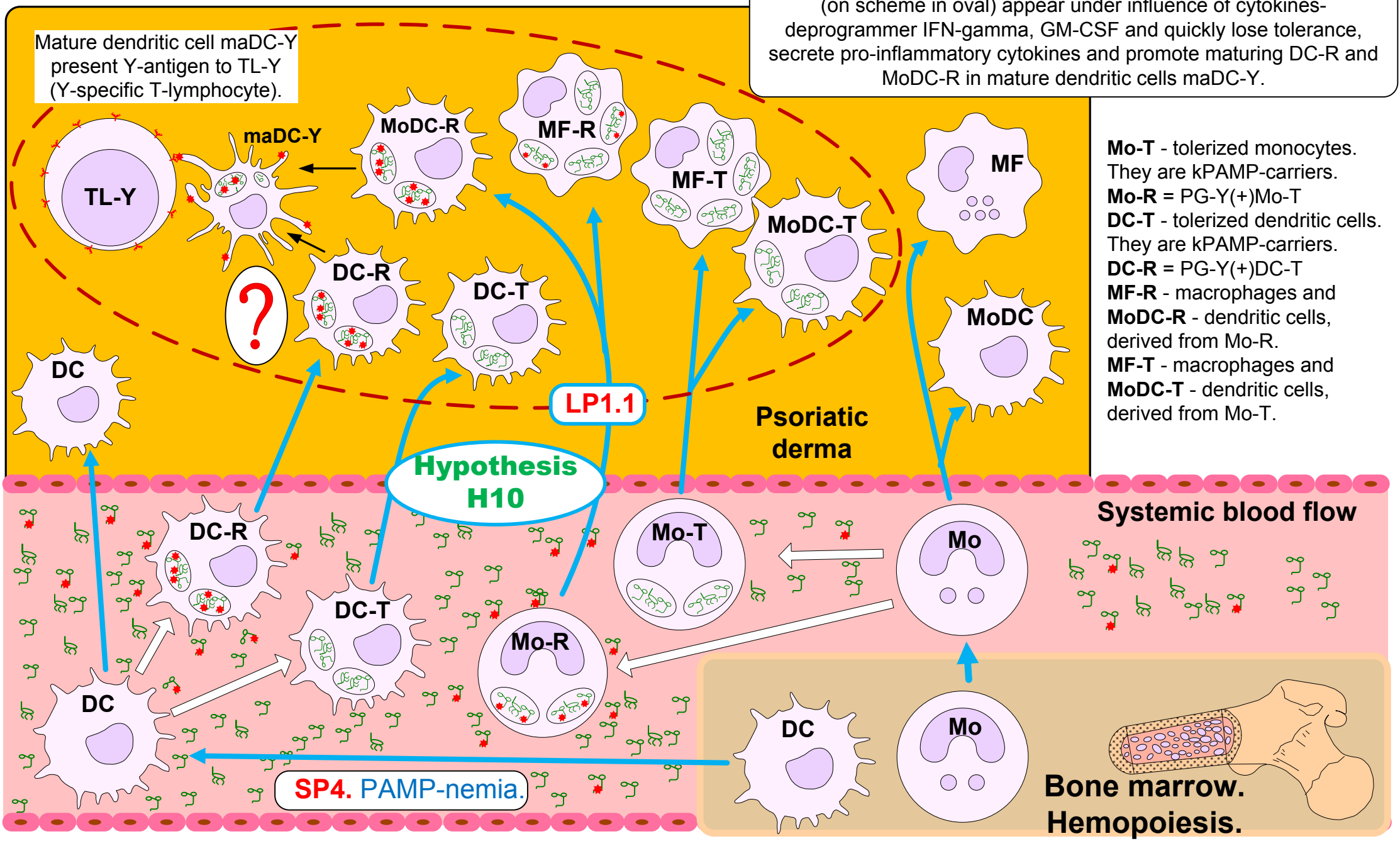
The formation in blood of fraction of tolerized monocytes Mo-T and dendritic cells DC-T (which are kPAMP-carriers) is possible, but not necessarily.

LP1a. Attraction of non-lymphocytic immunocytes from blood to skin.

Y-model. Attraction from blood and transformation of monocytes and dendritic cells in psoriatic derma.

Tolerized phagocytes attracted in inflamed derma from blood flow (on scheme in oval) appear under influence of cytokines-deprogrammer IFN-gamma, GM-CSF and quickly lose tolerance, secrete pro-inflammatory cytokines and promote maturing DC-R and MoDC-R in mature dendritic cells maDC-Y.

Mature dendritic cell maDC-Y present Y-antigen to TL-Y (Y-specific T-lymphocyte).



Mo-T - tolerized monocytes. They are kPAMP-carriers.
Mo-R = PG-Y(+)Mo-T
DC-T - tolerized dendritic cells. They are kPAMP-carriers.
DC-R = PG-Y(+)DC-T
MF-R - macrophages and **MoDC-R** - dendritic cells, derived from Mo-R.
MF-T - macrophages and **MoDC-T** - dendritic cells, derived from Mo-T.

YN-model. Attraction of neutrophils from blood and netosis some of them. Endocytosis and presentation Y-antigens lost by Neu-Y during netosis.

Psoriatic skin

NET - netotic products from Neu-Y and Neu

HBD-2

KC KC KC

KC - keratinocytes

AMP

hDNA, hRNA

LL37

LP3b

Complexes hDNA-LL37, hRNA-LL37

PDC – plasmacytoid dendritic cells

PDC

LP4

TL – any T-lymphocytes

IFN-alpha, TNF-alpha

TL

TL-Y

Neu - neutrophils

Neu-Y

some %

Neu

LP3a

Endocytosis of netotic products, including non-degraded LPS, PG, PG-Y and nhDNA ?

LPN6

DC

Mo

MF

DC-Y

LP6a

Mo-Y

LP6b

MoDC-Y

LP6c

maDC-Y

LP6c

LP8.1

Dendritic cells **DC** and monocytes **Mo** after such endocytosis can be transformed in **DC-Y** = PG-Y(+)DC and in **Mo-Y** = PG-Y(+)Mo and further in **MoDC-Y**.

Mature dendritic cell **maDC-Y** present to **TL-Y** (Y-specific T-lymphocyte) Y-antigen lost by **Neu-Y**.

LP1a

Hypothesis HN10

LP1a

Neu-Y

Neu

PAMP-load

Neu-Y = PG-Y(+)Neu

SPN4. PAMP-nemia.

Systemic blood flow

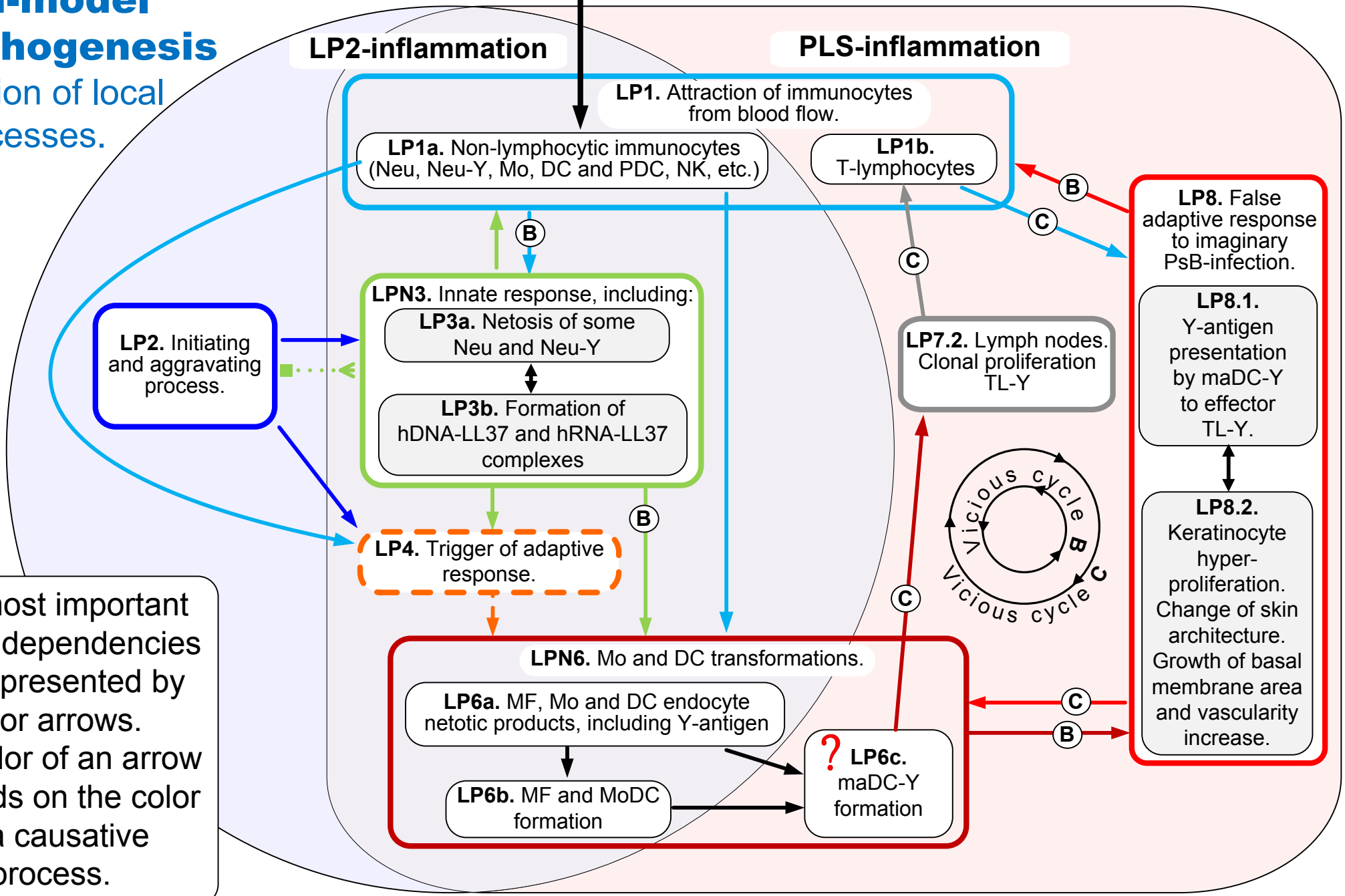
Neu

Bone marrow. Hemopoiesis.

YN-model of pathogenesis

Interaction of local processes.

Systemic psoriatic process SPPN.
Increased kPAMP-carriage and increased (PG-Y)-carriage of blood phagocytes.



The most important causal dependencies are represented by color arrows.
The color of an arrow depends on the color of a causative process.

? 4 hypotheses (H1-1, H2, HN3 and HN10) on check

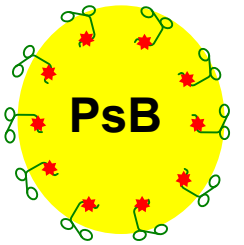
Systemic psoriatic process SPPN.

H1-1

One of two main causes of SPPN is increased macromolecular (including that for bacterial products) small intestine permeability.

H2

PsB have PG-Y - peptidoglycan with interpeptide bridges IB-Y, i.e. (L-Ala)-(L-Ala) and-or (L-Ser)-(L-Ala).
Y-antigen is part(s) of interpeptide bridge IB-Y.



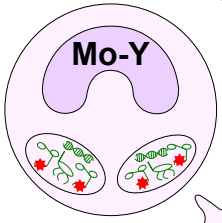
HN3

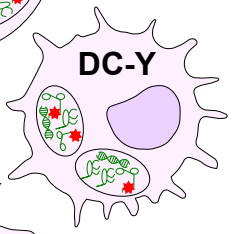
PAMP-nemia and (PG-Y)-nemia - these are main subprocesses
kPAMP are LPS, PG and bacDNA.

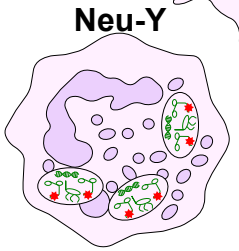
One of local processes.

HN10

Attraction of (PG-Y)+ blood phagocytes to skin from blood flow as an essential link of the vicious cycle.
Existence and severity of any psoriatic plaque is determined by income intensity of Y-antigen, carried by these blood phagocytes.



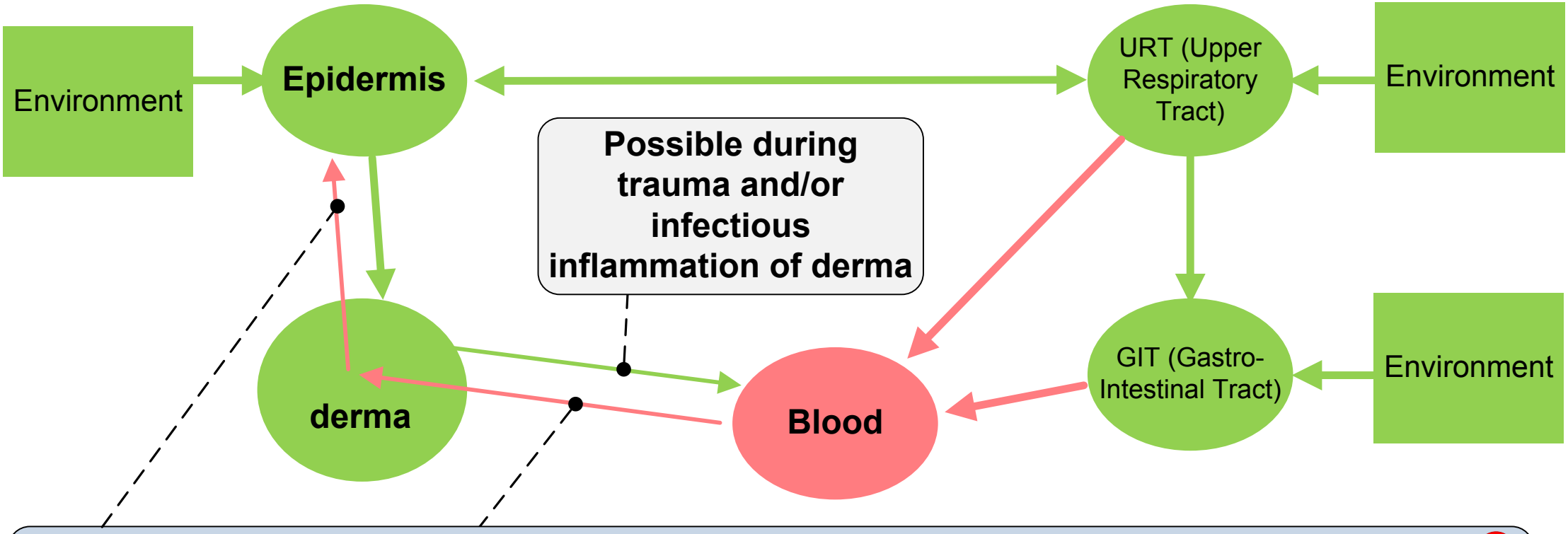




Hypotheses H2 and HN10 will be indirectly checked.

Presence and movement of non-host biomaterial between organs

Biomaterial	Presence	Movement
Bacteria and bacterial products (including bacDNA)	●	→
Only bacterial products (including bacDNA)	●	→

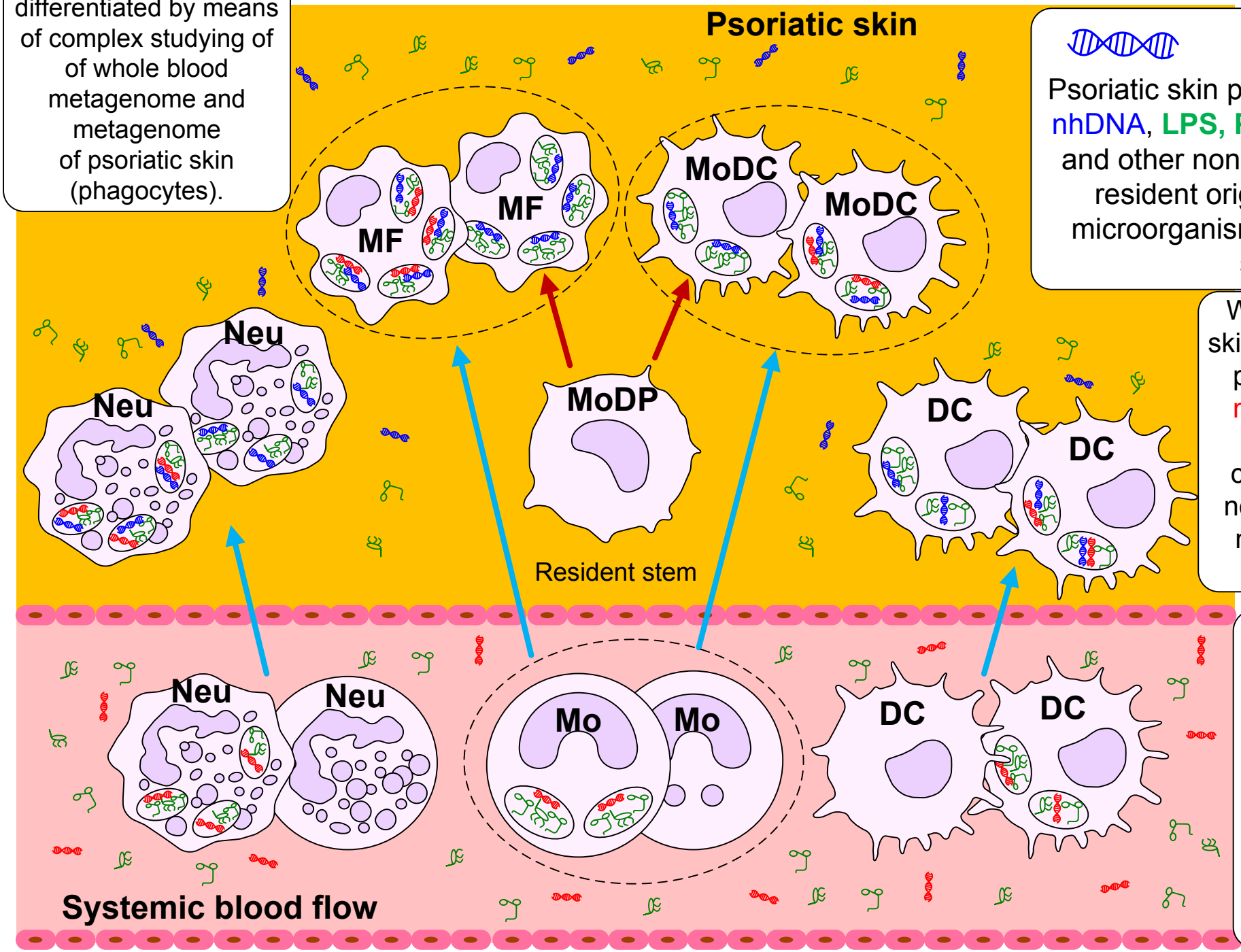


Hypothesis HN10-S (general).
Non-degraded non-host biomaterial moves to psoriatic skin in blood phagocytes. ?

Hypothesis HN10. Attraction of (PG-Y)+ blood phagocytes in skin from blood flow - necessary link of vicious cycle. Existence and severity of any psoriatic plaque is defined by intensity of Y-antigen income, carried by these blood phagocytes. ?

Non-host biomaterial comes to psoriatic skin inside blood phagocytes (hypothesis HN10-S).

nhDNA resident and nhDNA of non-resident origin can be differentiated by means of complex studying of of whole blood metagenome and metagenome of psoriatic skin (phagocytes).



Psoriatic skin phagocytes endocyte nhDNA, **LPS, PG** (including **PG-Y**) and other non-host biomaterial of resident origin (i.e. from any microorganisms living on and in skin).

WMS-tests of psoriatic skin (phagocytes) make it possible to determine nhDNA concentration and to estimate concentration of other non-host biomaterial of non-resident origin in psoriatic skin.

nhDNA, **LPS, PG** (including **PG-Y**) and other non-host biomaterial gets into psoriatic skin from blood flow inside blood phagocytes

Metagenomes* of blood and psoriatic skin. Research project.

Section 2.

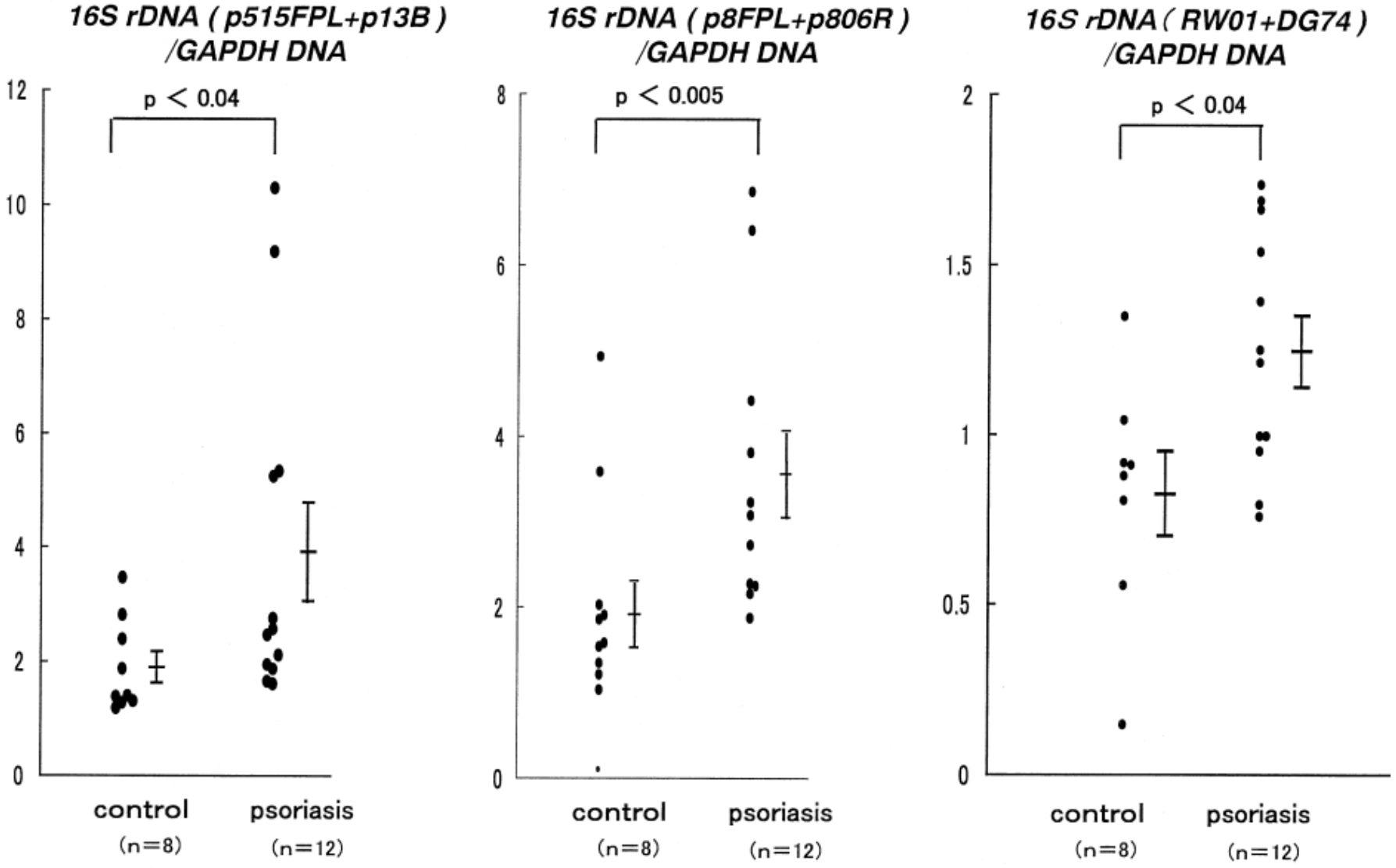
Metagenomic sequencing.

Blood metagenome.

Skin metagenome.

* Metagenome is a complex of all nhDNA (non-host DNA, that is, non-human here) contained in a biomaterial. nhDNA is a bacterial, archean, fungal, helminthic, viral, phage, etc. DNA.

Total bacDNA in blood monocytes of PP and HP
(16S-test, Okubo 2002)



The number of 16S rDNA copies in blood monocytes of PP (psoriatic patients) and HP (healthy persons) in the form of relation to copy number of human gene GAPDH. Values for each pair of primers are considerably increased in PP compared to HP.

Fig.2 from Okubo Y, Oki N, Takeda H, Amaya M. et al. Increased microorganisms DNA levels in peripheral blood monocytes from psoriatic patients using PCR with universal ribosomal RNA primers. J Dermatol. 2002 Sep;29(9):547-55. PMID 12392062.

BacDNA in blood plasma of psoriatic patients (16S-test, Munz 2010)

Table 1 Summary of bacterial genera identified in psoriasis patients

Bacterial genus	GP ($n = 7$)	CPP/GF ($n = 7$)	CPP ($n = 6$)
Streptococcus sp.	6[a]	1	1
Staphylococcus sp.	–	5	4
Propionibacterium sp.	–	–	1
Bacillus sp.	–	1	–
Exiguobacterium sp.	1	–	–

a - number of patients in whom this bacterial genus is found;
GP - Guttage psoriasis
CPP - Chronic plaque psoriasis.
BacDNA was found in all 20 PP and in none out of 12 HP.

Munz OH, Sela S, Baker BS et al. Evidence for the presence of bacteria in the blood of psoriasis patients. Arch Dermatol Res. 2010 Sep;302(7):495-8. 20607546.

Pathogens identified by cultural method (BC) and NGS (WMS-test) in blood plasma (Long 2016)

Type	Pathogen	Strain identified		
		BC (+)	NGS (+)	BC and NGS
Gram-positive bacteria	*Enterococcus faecalis*	2	1	2
	Enterococcus faecium	1	3	3
	Lactococcus lactis	0	1	1
	Staphylococcus aureus	1	2	2
Gram-negative bacteria	*Acinetobacter baumannii*	2	1	2
	Aeromonas hydrophila	0	1	1
	Bacteroides fragilis	1	1	1
	Citrobacter freundii	1	1	1
	Escherichia coli	0	1	1
	Klebsiella pneumoniae	1	3	3
	Pseudomonas aeruginosa	1	2	2
Fungi	*Candida albicans*	1	0	1
	Total	11	17	20

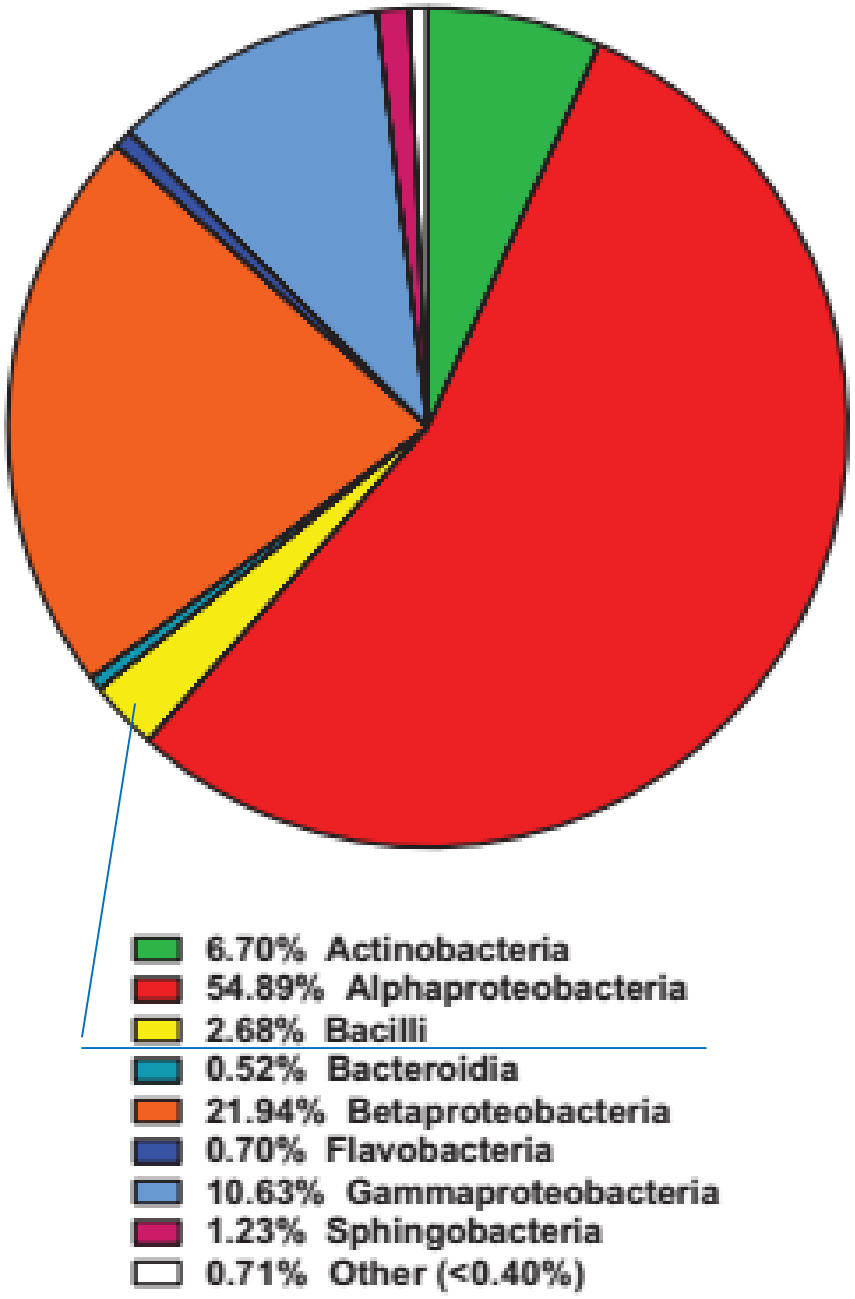
Quantity of cultured and mapped (NGS) species of bacteria and fungi at 78 patients and 10 PP.

Table 1 from Long Y, Zhang Y, Gong Y at al. Diagnosis of Sepsis with Cell-free DNA by Next-Generation Sequencing Technology in ICU Patients. Arch Med Res. 2016 Jul;47(5):365-371. 27751370..

BacDNA in whole blood (30 HP, 16S-test, Paisse 2016)

Representation of bacDNA (bacterial DNA) found in whole blood of 30 HP (donors) by 16S-test.
bacDNA concentration constituted
$4.2*10^7$ (16S copies)/(ml of whole blood)
on average.
The greatest part (93.7%) is found in buffy coat - fraction of leukocytes and platelets.
Bacterial class structure is demonstrated.

Within NCS1 it is expected to apply WMS-test (whole metagenomic sequencing) of whole blood which will enable us to establish the structure of nhDNA (including bacterial nhDNA) to within species.



Fragment of Fig.2 from Païssé S, Valle C, Servant F. et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. Transfusion. 2016 May;56(5):1138-47. 26865079.

The name of the class, species of bacteria presumed psoriagenic belong to, is underlined.

Characteristics of blood plasma (7 patients with sepsis, 12 HP, WMS-test, Grumaz 2016)

Table 1 Patient characteristics, cfDNA concentration, and sequencing statistics (cfDNA = cell-free DNA)

	ID	Time	Sex	Age (years)	cfDNA (ng/ml plasma)	Sequencing depth	Human reads (%)	Unmapped (%)	Classified (%)
Patients with sepsis	S9	T0	M	82	120.59	30,650,143	92.90	7.10	28.90
	S10	T0	M	68	307.83	27,199,593	98.70	1.30	2.85
	S11	T0	M	62	805.50	27,073,879	93.61	6.39	20.73
	S19	T0	F	62	101.30	26,892,684	98.45	1.55	4.75
	S23	T0	M	79	146.70	24,917,032	97.12	2.88	3.85
	S26	T0	M	66	1088.90	32,529,889	96.60	3.40	3.24
	S60	T0	F	70	70.29	27,381,853	97.10	2.90	4.40
			Average S T0	70	377.30	28,092,153	96.36	3.64	9.82
			Average S all	70	197.23	25,960,730	97.79	2.21	4.24
Healthy persons	V5		M	24	35.80	34,203,815	81.90	18.10	12.38
	V6		M	29	27.40	30,000,000	98.96	1.04	2.25
	V7		F	22	76.40	21,004,601	96.58	3.42	2.35
	V13		F	26	23.50	24,449,232	98.09	1.91	3.26
	V14		M	28	38.60	37,971,559	97.42	2.58	1.79
	V15		M	27	166.80	24,505,696	97.60	2.40	2.88
	V16		F	29	70.60	27,220,925	97.06	2.94	2.67
	V17		M	26	28.40	20,225,374	98.61	1.39	3.30
	V18		M	28	48.80	19,157,938	98.14	1.86	2.46
	V19		F	31	33.40	25,776,920	97.08	2.92	2.87
	V21		M	22	67.30	25,220,391	97.72	2.28	2.51
	V22		M	25	48.20	30,000,000	99.15	0.85	3.25
			Average V	26	55.43	26,644,704	96.52	3.48	3.50

Fragment of Table 1 from Grumaz S, Stevens P, Grumaz C. et al. Next-generation sequencing diagnostics of bacteremia in septic patients. Genome Med. 2016 Jul 1;8(1):73. 27368373.

Blood plasma metagenome (12 HP, WMS-test, Grumaz 2016)

Species	% of reads	Species	% of reads
Micrococcus luteus	35.14%	Streptococcus oralis	0.35%
Staphylococcus epidermidis	15.93%	Streptococcus sanguinis	0.34%
Rhodococcus erythropolis	2.99%	Pseudomonas fluorescens	0.32%
Gardnerella vaginalis	2.87%	Alicycliphilus denitrificans	0.27%
Staphylococcus warneri	2.85%	beta proteobacterium CB	0.28%
Stenotrophomonas maltophilia	1.92%	Bacteroides vulgatus	0.20%
Lactobacillus sakei	2.05%	Staphylococcus saprophyticus	0.25%
Escherichia coli	0.25%	Klebsiella pneumoniae	0.21%
Acinetobacter baumannii	1.51%	Variovorax paradoxus	0.25%
Kytococcus sedentarius	1.37%	Acidovorax ebreus	0.24%
Acidovorax sp. KKS102	1.30%	Staphylococcus pasteuri	0.24%
Streptococcus parasanguinis	1.15%	Burkholderia xenovorans	0.23%
Rothia mucilaginosa	1.08%	Bradyrhizobium sp. BTAi1	0.24%
Rothia dentocariosa	1.14%	Legionella pneumophila	0.23%
Leuconostoc carnosum	0.94%	Delftia sp. Cs1-4	0.22%
Streptococcus salivarius	0.90%	Corynebacterium variabile	0.22%
Streptococcus thermophilus	0.87%	Propionibacterium avidum	0.18%
Staphylococcus haemolyticus	0.87%	Methylobacterium extorquens	0.20%
Pseudomonas aeruginosa	0.47%	Fusobacterium nucleatum	0.11%
Burkholderia phytofirmans	0.73%	Streptococcus gordonii	0.17%
Lactococcus lactis	0.67%	Bacillus megaterium	0.17%
Enterobacter cloacae	0.71%	Anaerococcus prevotii	0.16%
Pseudomonas sp. TKP	0.59%	Eubacterium rectale	0.11%
Pseudomonas stutzeri	0.64%	Ralstonia pickettii	0.15%
Staphylococcus aureus	0.51%	Thermus scotoductus	0.14%
Haemophilus parainfluenzae	0.57%	Candidatus Saccharimonas aal	0.14%
Cupriavidus metallidurans	0.12%	Kocuria rhizophila	0.14%
Comamonas testosteroni	0.45%	Bifidobacterium thermophilum	0.14%
Pseudomonas putida	0.47%	Methylobacterium radiotoleran	0.14%
Acidovorax sp. JS42	0.50%	Streptococcus pseudopneumon	0.14%
Delftia acidovorans	0.45%	Corynebacterium aurimucosun	0.12%
Veillonella parvula	0.44%	Pediococcus pentosaceus	0.13%
Lactobacillus crispatus	0.42%	Leuconostoc mesenteroides	0.11%
Streptococcus mitis	0.42%	Cupriavidus necator	0.11%
Pseudomonas resinovorans	0.37%	Collimonas fungivorans	0.11%
Finegoldia magna	0.37%	Burkholderia lata	0.10%
Pseudomonas mendocina	0.30%	Xanthobacter autotrophicus	0.10%
Streptococcus pneumoniae	0.36%	Rhizobium sp. IRBG74	0.10%
Prevotella melaninogenica	0.35%	Moraxella catarrhalis	0.10%

Species for which the percentage of reads is > 0.1% of the total number.
(P.acnes - is excluded as skin contaminant .)

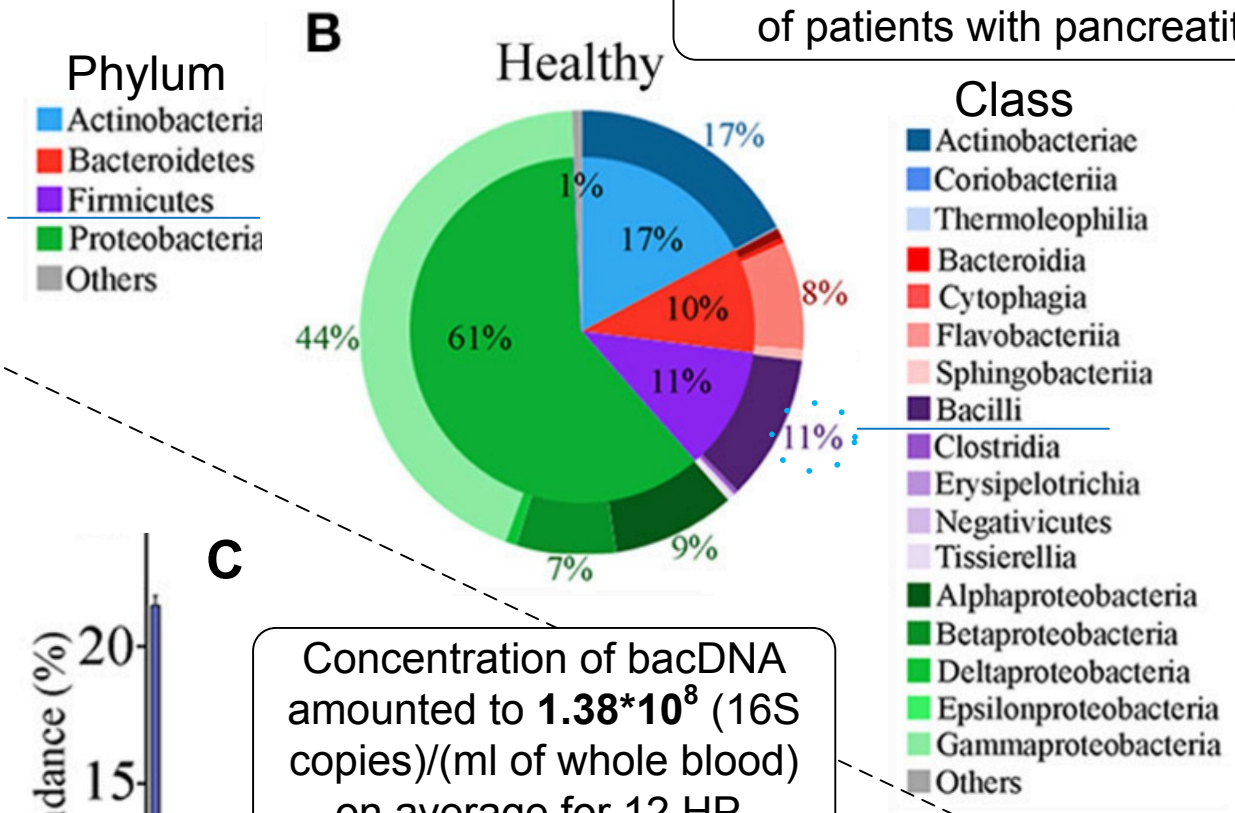
Among them there are species presumed psoriagenic.

Streptococcus parasanguinis	1.15%
Leuconostoc carnosum	0.94%
Streptococcus salivarius	0.90%
Streptococcus thermophilus	0.87%
Streptococcus mitis	0.42%
Streptococcus pneumoniae	0.36%
Streptococcus oralis	0.35%
Streptococcus sanguinis	0.34%
Streptococcus gordonii	0.17%
Streptococcus pseudopneumoniae	0.14%
Leuconostoc mesenteroides	0.11%
Total	5.75%

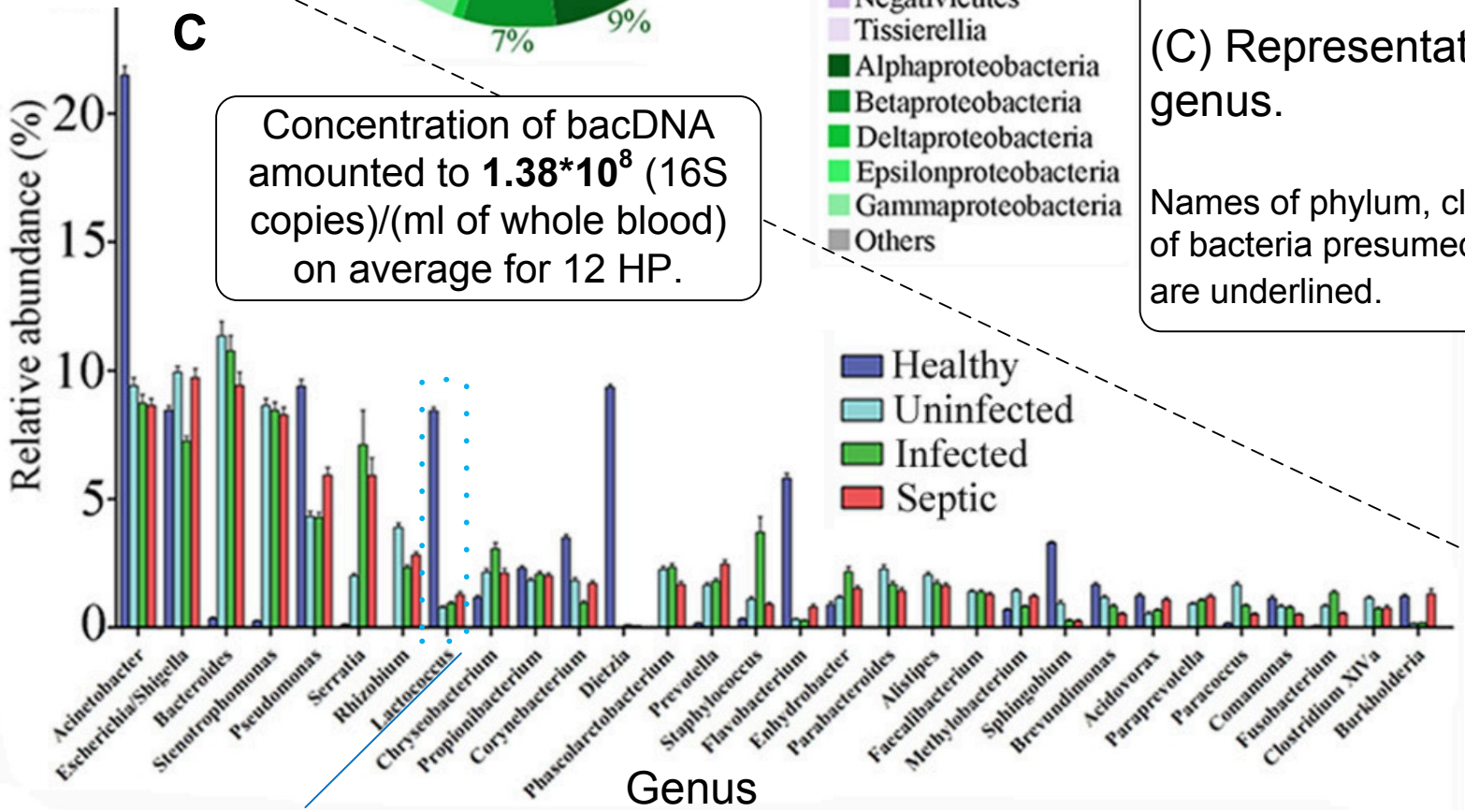
Selection from «Additional file 3: Table S6. Total read counts per sample and species» из Grumaz S, Stevens P, Grumaz C. et al. Next-generation sequencing diagnostics of bacteremia in septic patients. Genome Med. 2016 Jul 1;8(1):73. 27368373.

BacDNA in whole blood (12 HP, 16S-test, Li 2018)

12 healthy patients were examined along with three groups of patients with pancreatitis (uninfected, infected, septic)



Concentration of bacDNA amounted to **1.38*10^8** (16S copies)/(ml of whole blood) on average for 12 HP.



Characteristics of bacterial blood metagenome, presumably coming from GIT.

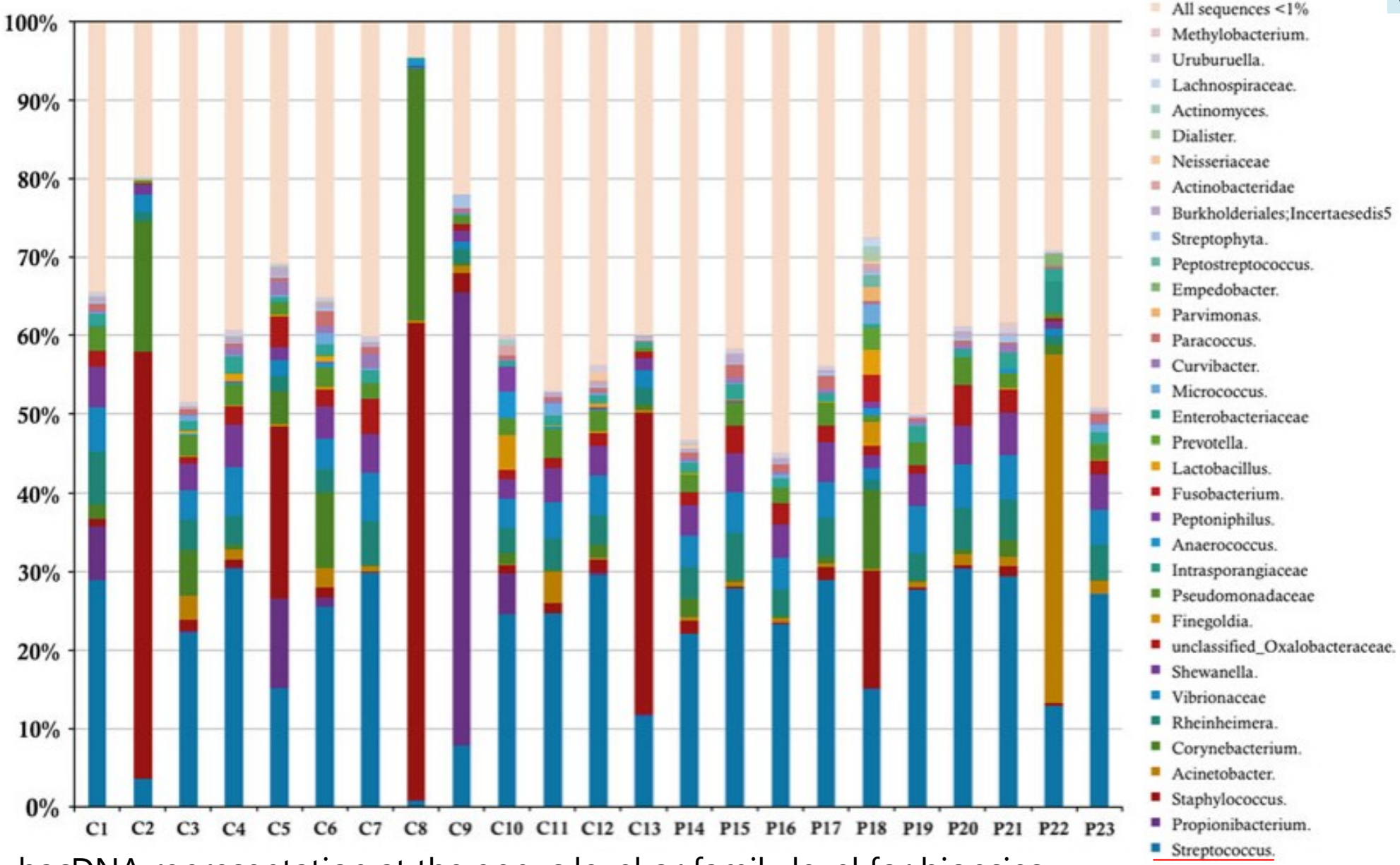
(B) Representation chart of bacterial genome for phylums and classes.

(C) Representation of 30 main bacterial genus.

Names of phylum, class and genus, which species of bacteria presumed psoriagenic belong to, are underlined.

Fragment Fig.1 from
Li Q, Wang C, Tang C, Zhao X, He Q, Li J. Identification and Characterization of Blood and Neutrophil-Associated Microbiomes in Patients with Severe Acute Pancreatitis Using Next-Generation Sequencing. Front Cell Infect Microbiol. 2018 Jan 23;8:5. 29423379.

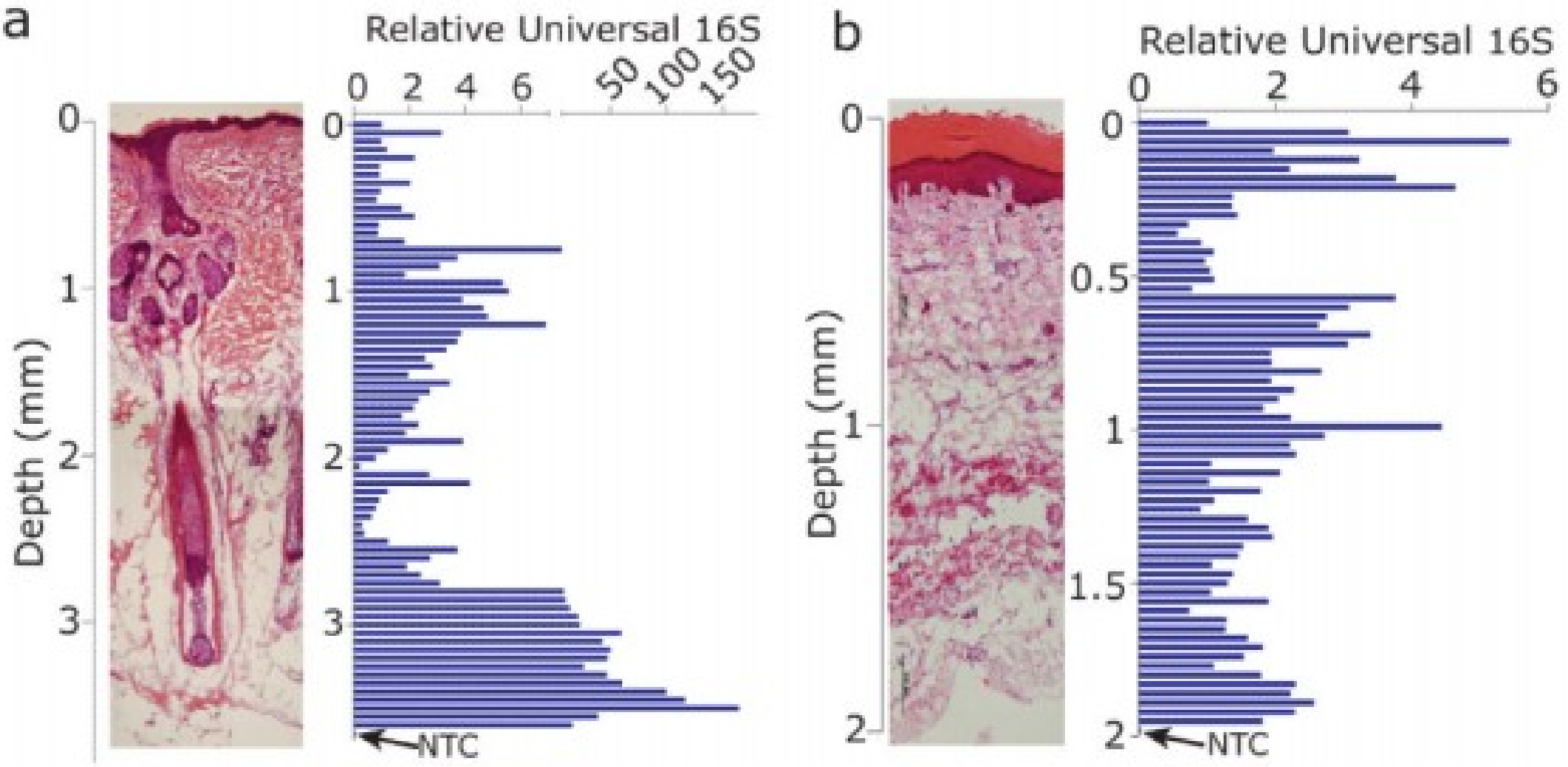
Bacterial DNA in psoriatic and healthy skin (10 PP, 12 HP, 16S-test, Fahlen 2012)



bacDNA representation at the genus level or family level for biopsies of healthy (C1-C13) and psoriatic (P14-P23) skin.

Fig.5 from Fahlen A, Engstrand L, Baker BS, Powles A, Fry L. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. Arch Dermatol Res. 2012 Jan;304(1):15-22. 22065152.

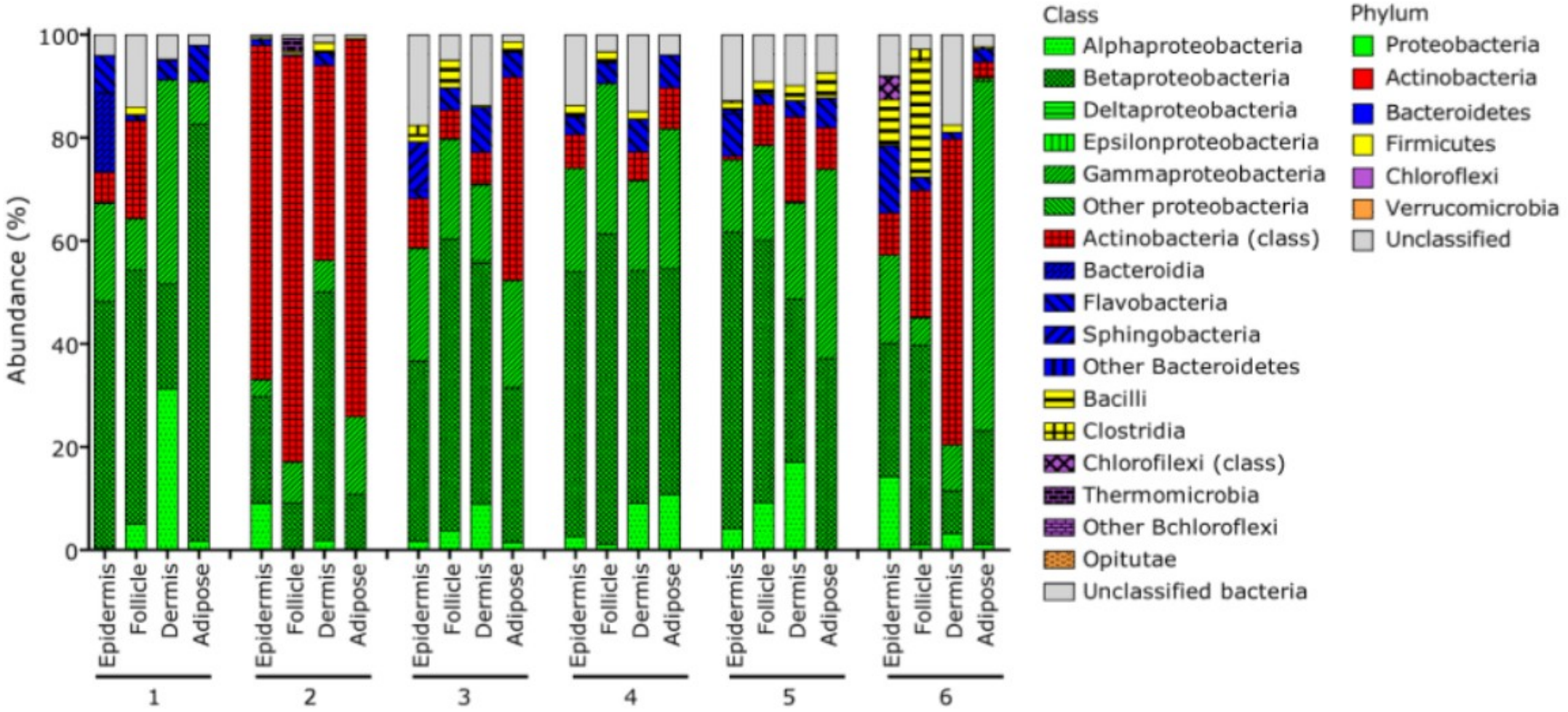
Bacterial DNA in epidermis and derma of non-psoriatic patients (16S-test, Nakatsuji 2013) - 1



BacDNA in normal skin is found at 2-3 mm depth
(a - face site with hair follicle, b - palmar site).

Fragment of Fig.1 from Nakatsuji T, Chiang HI, Jiang SB. The microbiome extends to subepidermal compartments of normal skin. Nat Commun. 2013;4:1431. 23385576.

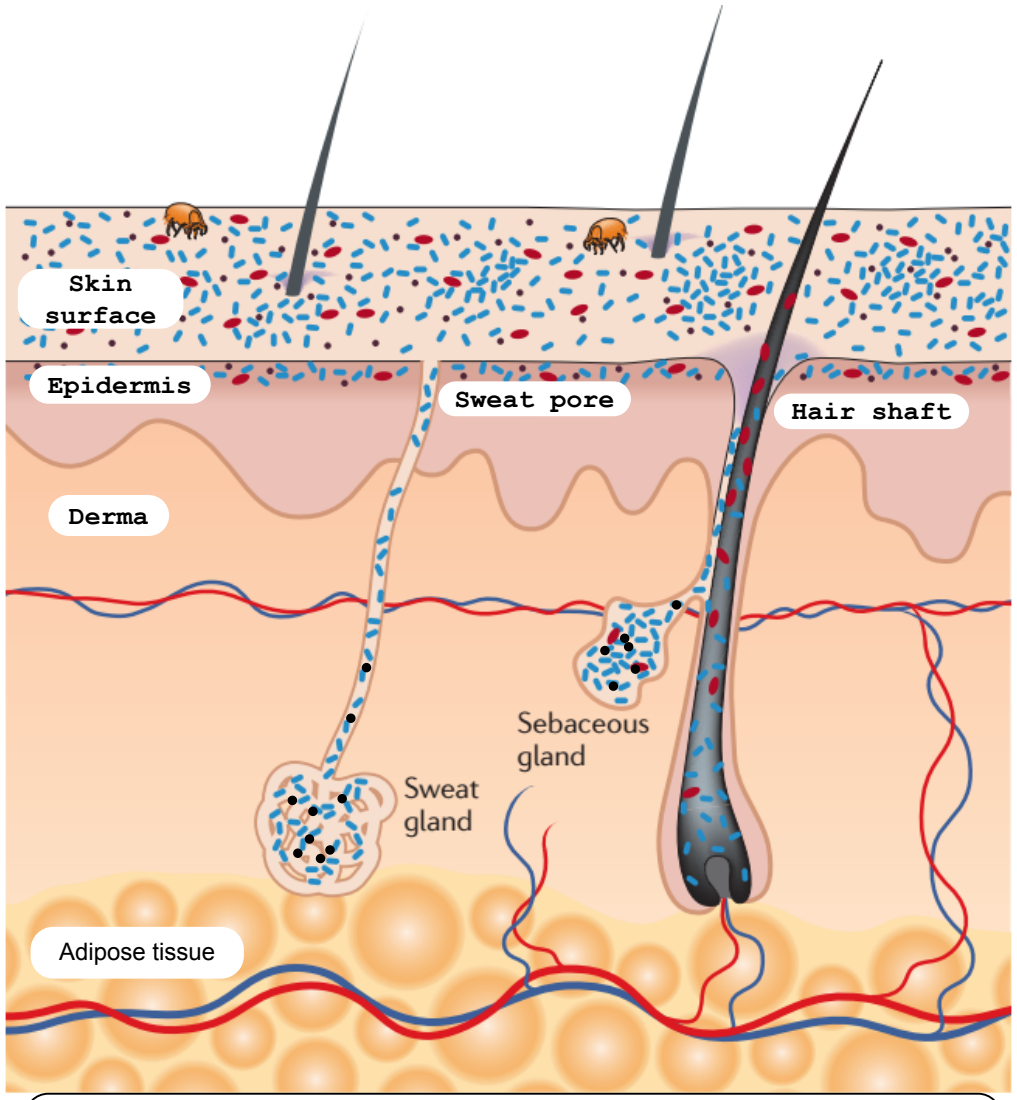
Bacterial DNA in epidermis and derma of non-psoriatic patients (16S-test, Nakatsuji 2013) - 2



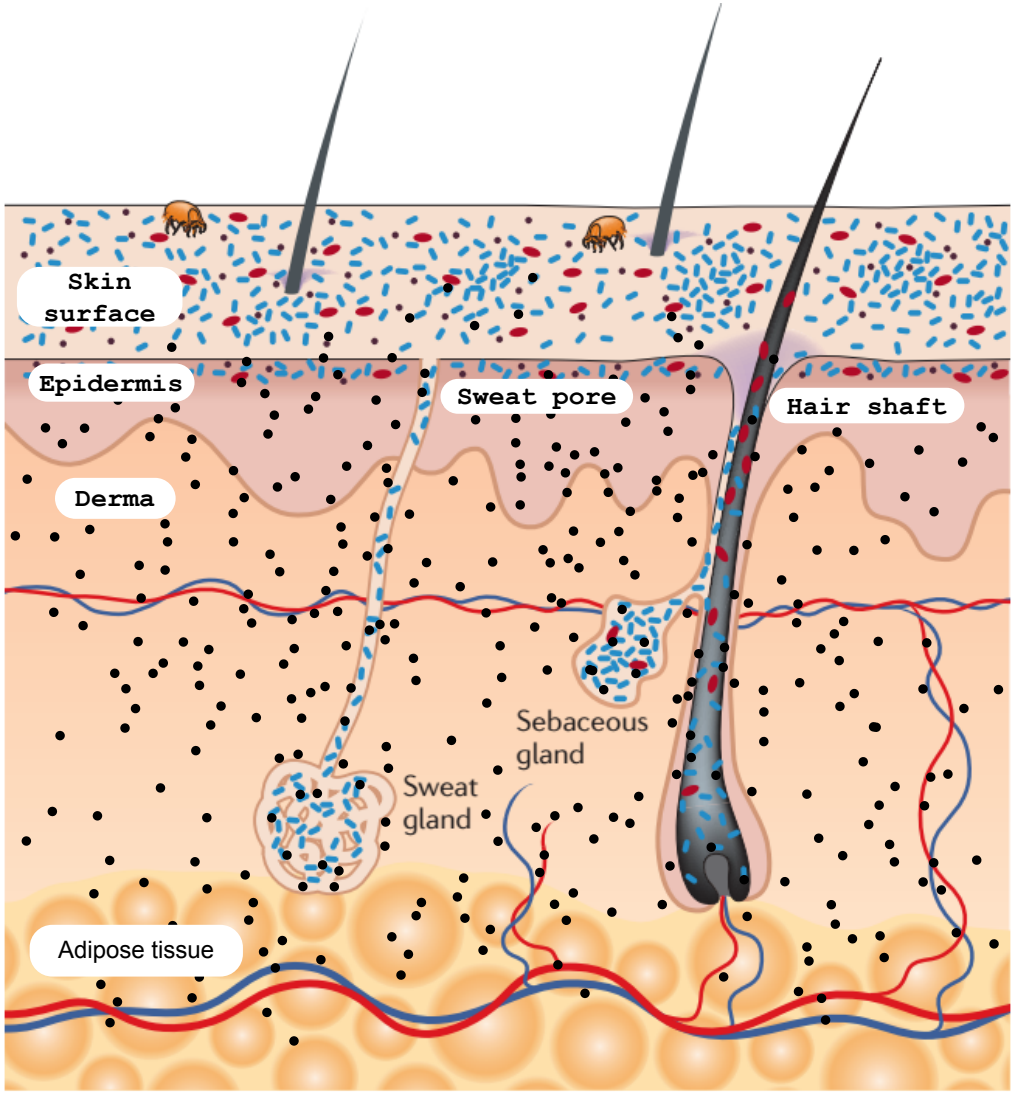
Variety of skin microbiome at class level. 16S-test of 4 parts of skin biopsies.
Representation is given for each parts: epidermis, follicular derma, derma without follicles (dermis) and dermal adipose tissue. Biopsies (1-6) were taken from non-psoriatic patients.
Results (at order level) are presented in table form in additional materials to the article.

Fragment of Fig.3 from Nakatsuji T, Chiang HI, Jiang SB. The microbiome extends to subepidermal compartments of normal skin. Nat Commun. 2013;4:1431. 23385576.

Microorganisms (including bacteria and bacDNA) in healthy skin. Assumptions and facts.



Before 2013 bacDNA was supposed to be present only in epidermis or in sweat or sebaceous glands.



Due to Nakatsuji T. et al. (2013) it become clear that bacDNA is present at all skin layers.

Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2011 Apr;9(4):244-53. 21407241.

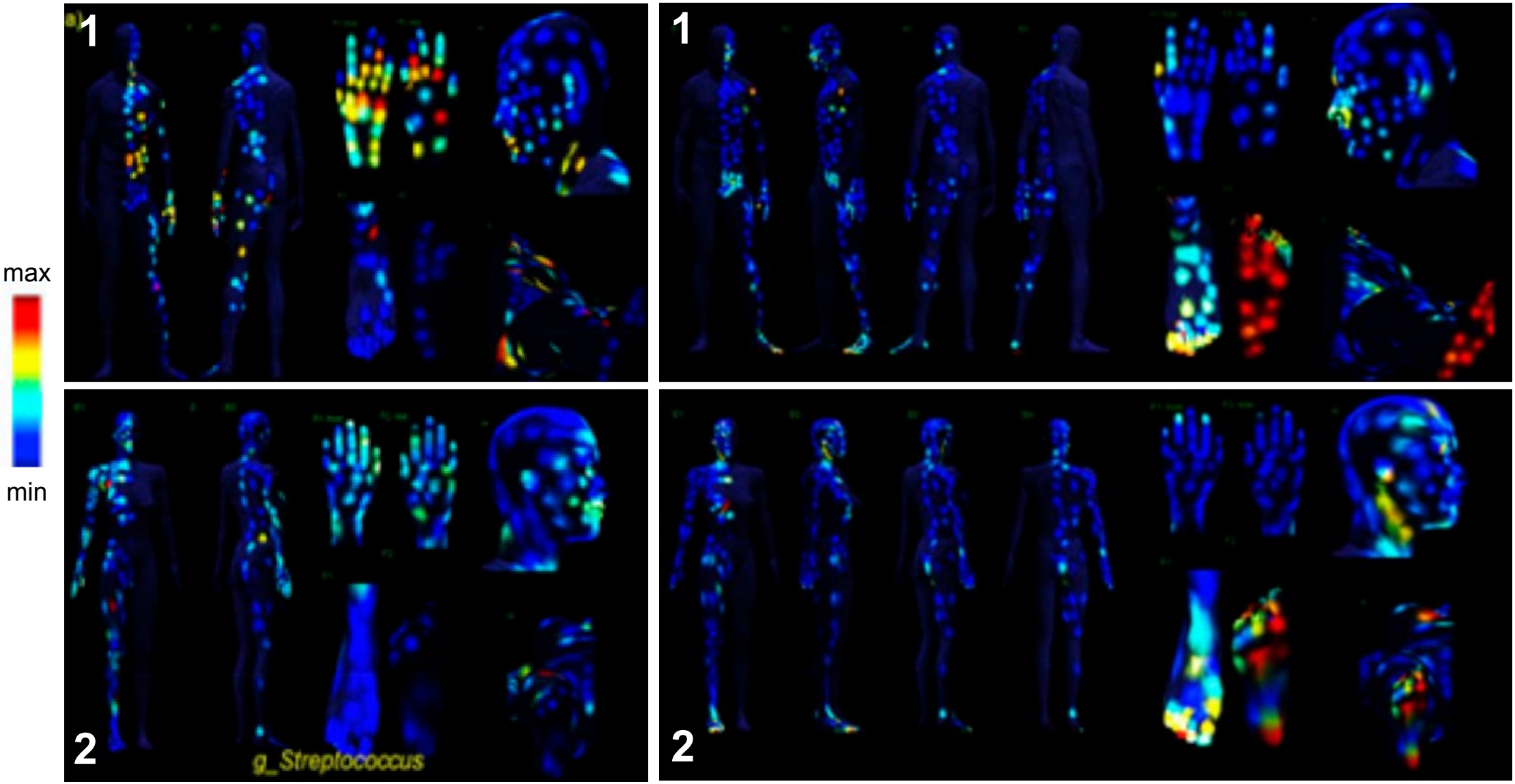
Virus • Bacterium • Fungus Mite

Nakatsuji T, Chiang HI, Jiang SB. The microbiome extends to subepidermal compartments of normal skin. Nat Commun. 2013;4:1431. 23385576.

Bacterial DNA on healthy skin men (1) and women (2). (16S-test, Bouslimani 2015)

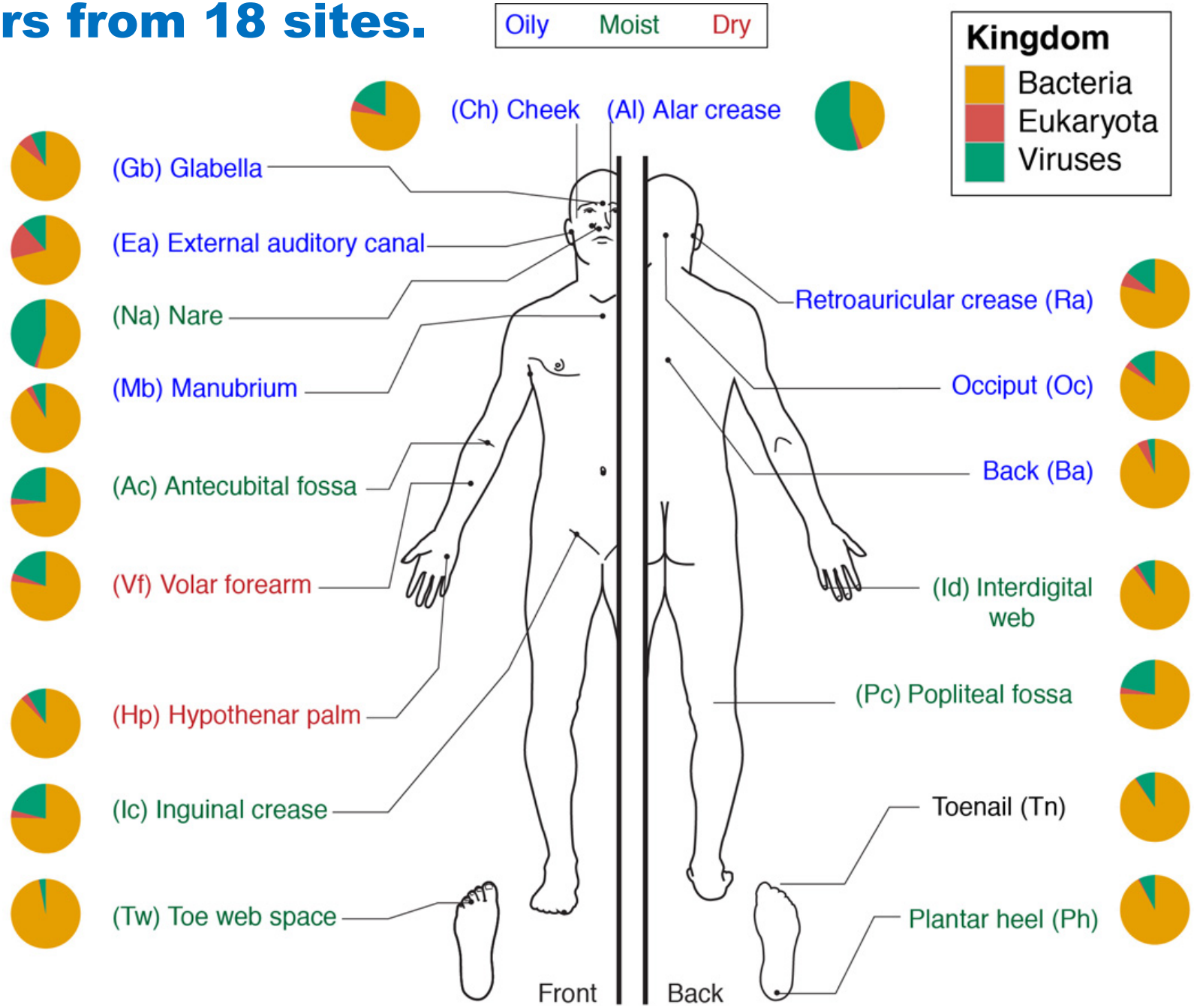
Streptococcus sp.

Staphylococcus sp.



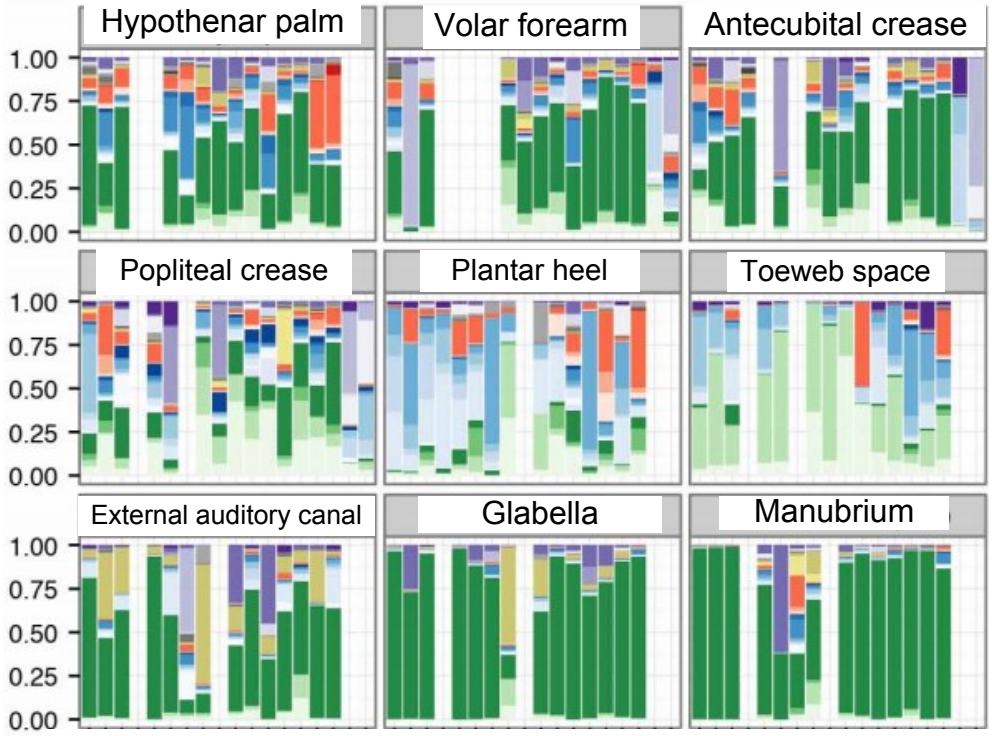
Bouslimani A, Porto C, Rath CM et al. Molecular cartography of the human skin surface in 3D. Proc Natl Acad Sci U S A. 2015 Apr 28;112(17):E2120-9. 25825778.

Skin biogeography (15 HP, WMS-test, Oh 2014). Smears from 18 sites.

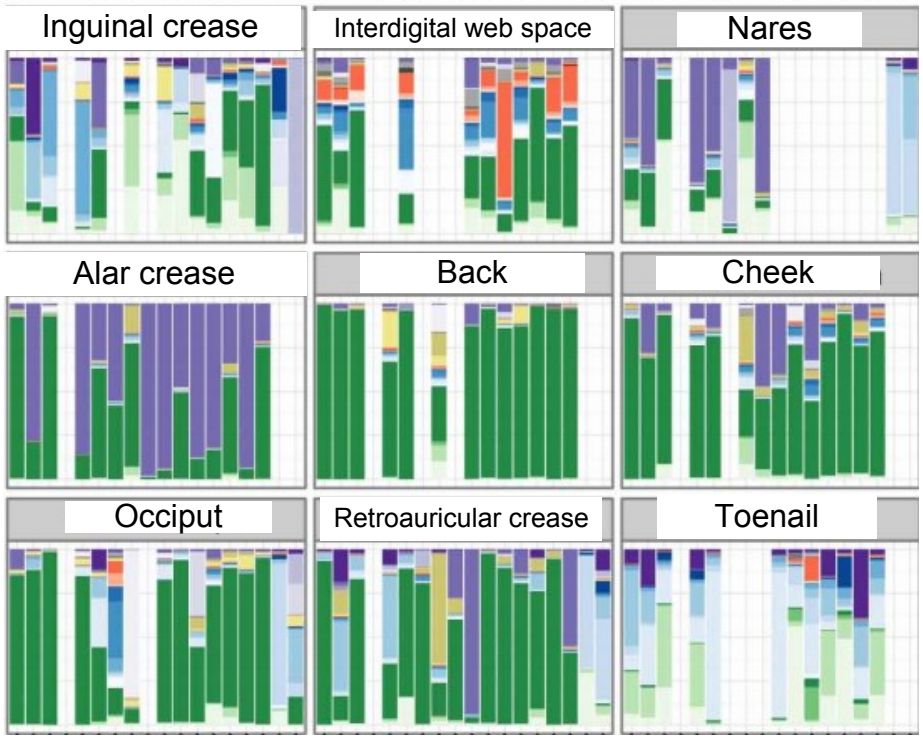


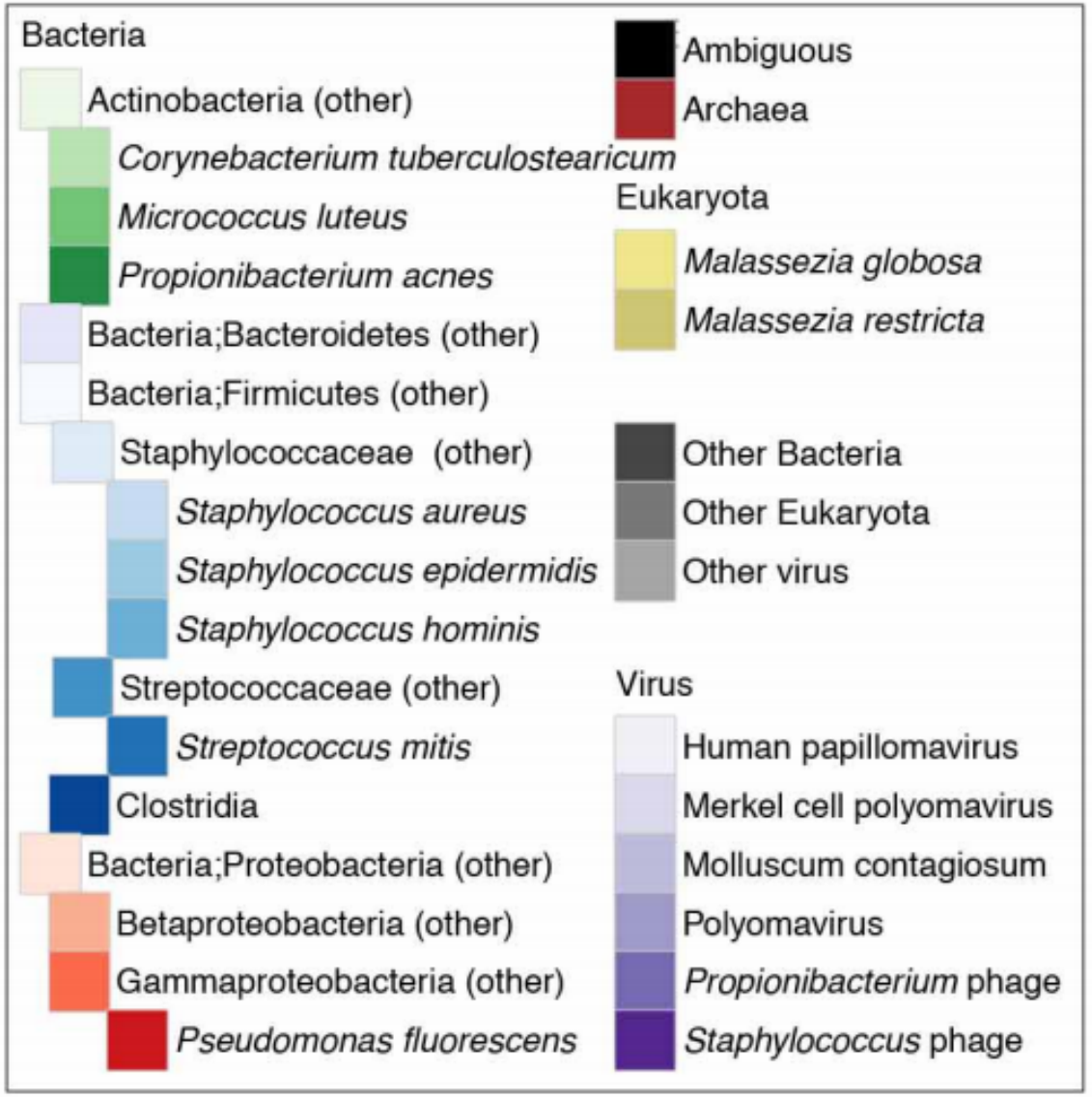
Oh J, Byrd AL, Deming C. et al. Biogeography and individuality shape function in the human skin metagenome.Nature. 2014 Oct 2;514(7520):59-64. 25279917.

Skin biogeography (15 HP, WMS-test, Oh 2014). Main results.



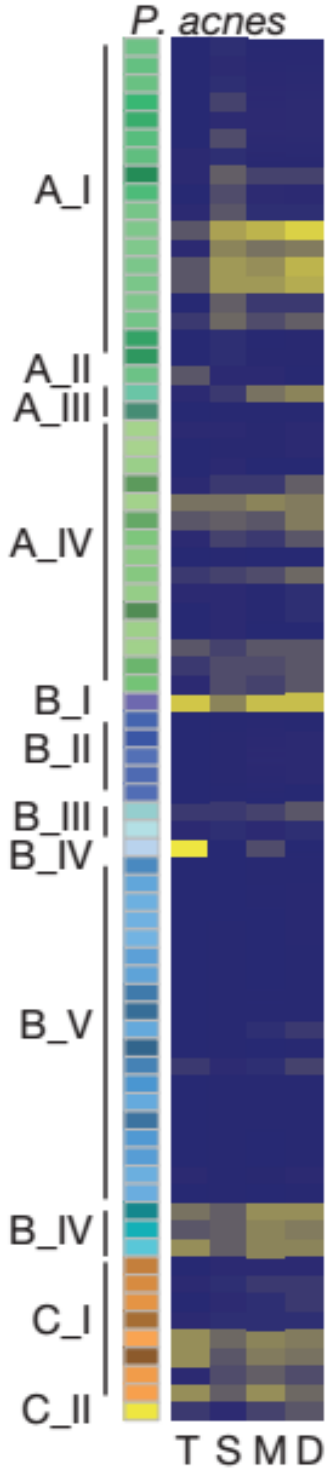
18 sites for each of 15 HP

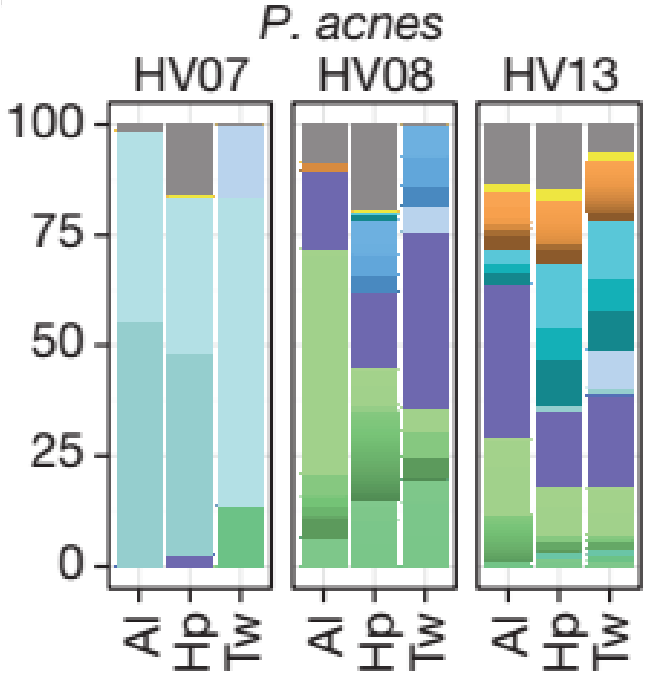


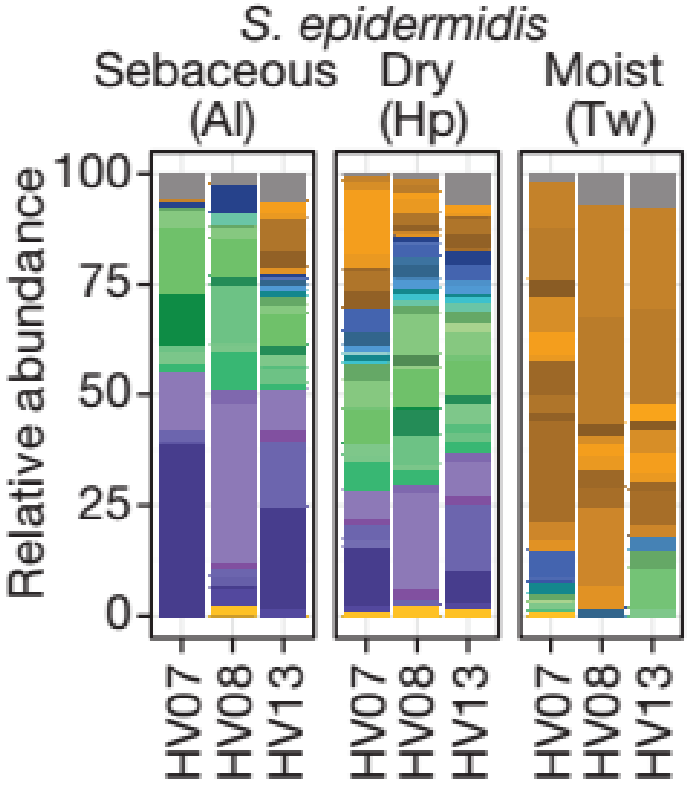


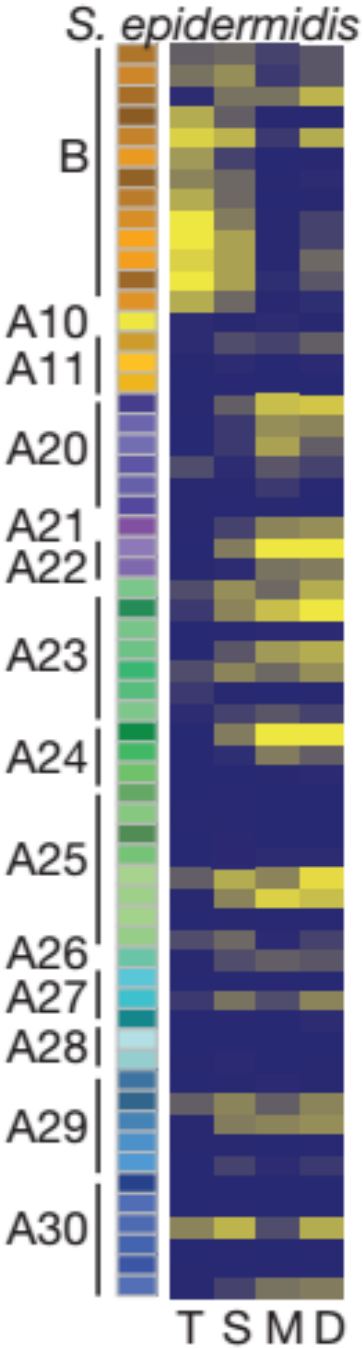
Oh J, Byrd AL, Deming C. et al. Biogeography and individuality shape function in the human skin metagenome.Nature. 2014 Oct 2;514(7520):59-64. 25279917.

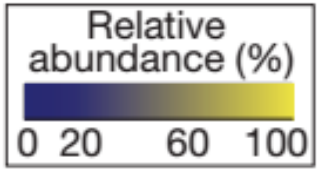
Skin Biogeography (15 HP, WMS-test, Oh 2014). Specification for two widespread species (P.acnes and Staph.epidermidis) to within strains.







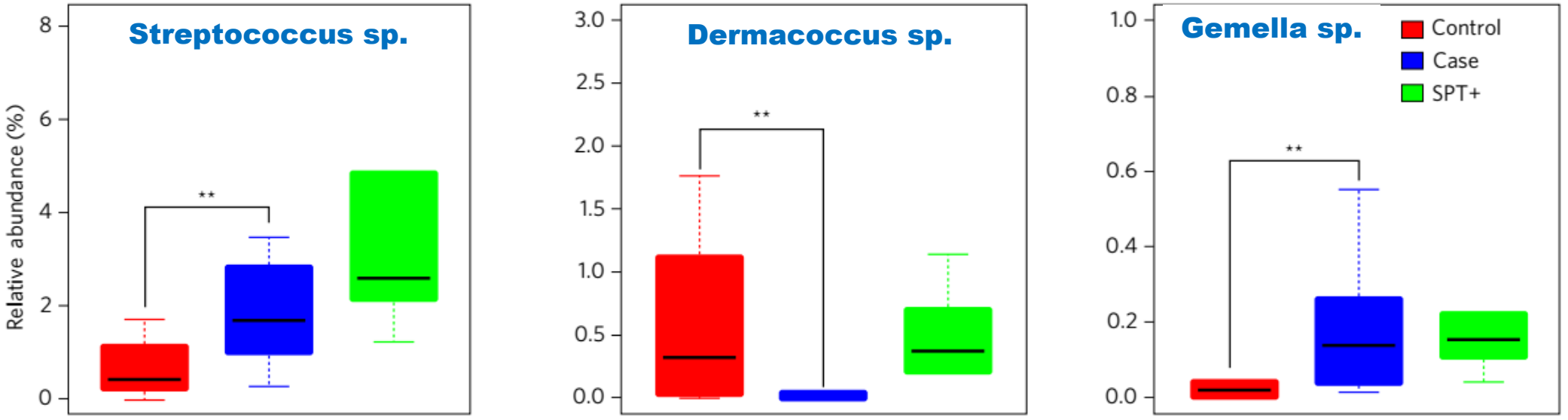




Al - Alar crease
Hp - Hypothenar palm
Tw - Toeweb space

HV07, HV08, HV13 - codes of patients

Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of several genera.

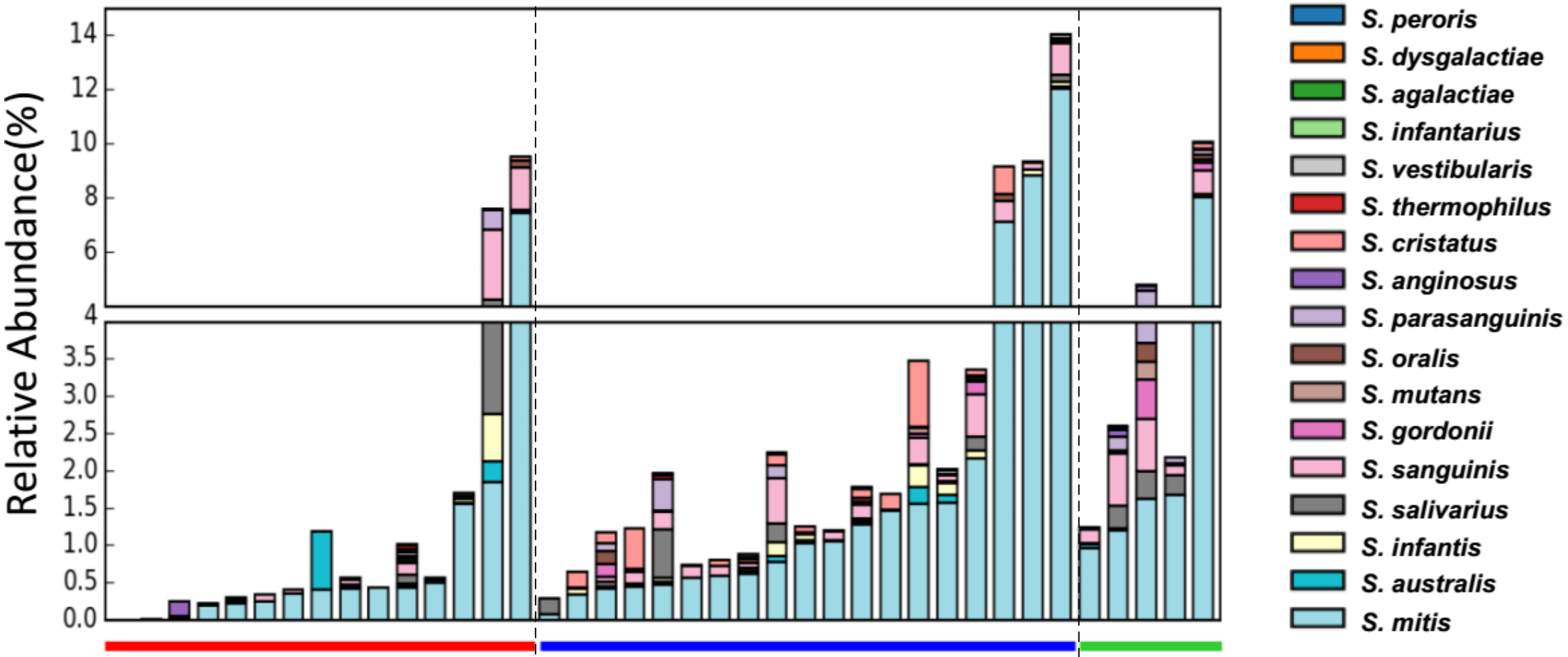


Control. 15 HP with negative skin prick test.
Case. 19 AtD+ (patients with history of atopic dermatitis). Healthy skin.
SPT+. 5 HP with positive skin prick test.

At everyone two smears (from right and left antecubital fossae) by tape stripping. Results of two WMS-tests were averaged.

Chng KR, Tay AS, Li C. et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nat Microbiol. 2016 Jul 11;1(9):16106. 27562258.

Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of Streptococcus sp.



Control. 15 HP with negative skin prick test.

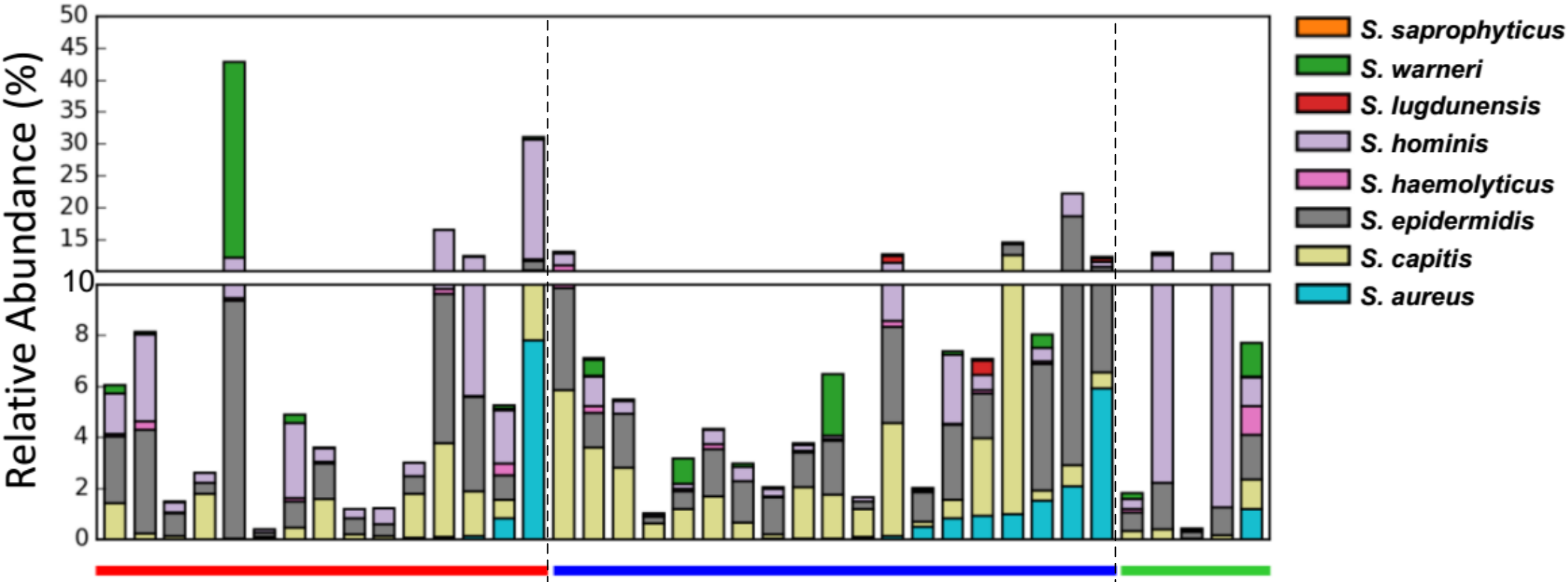
Case. 19 AtD+ (patients with history of atopic dermatitis). Healthy skin.

SPT+. 5 HP with positive skin prick test.

From each person two smears (from right and left antecubital fossae) were taken by tape stripping. Two WMS-tests results were averaged.

Chng KR, Tay AS, Li C. et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nat Microbiol. 2016 Jul 11;1(9):16106. 27562258.

Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of Staphylococcus sp.



Control. 15 HP with negative skin prick test.

Case. 19 AtD+ (patients with history of atopic dermatitis). Healthy skin.

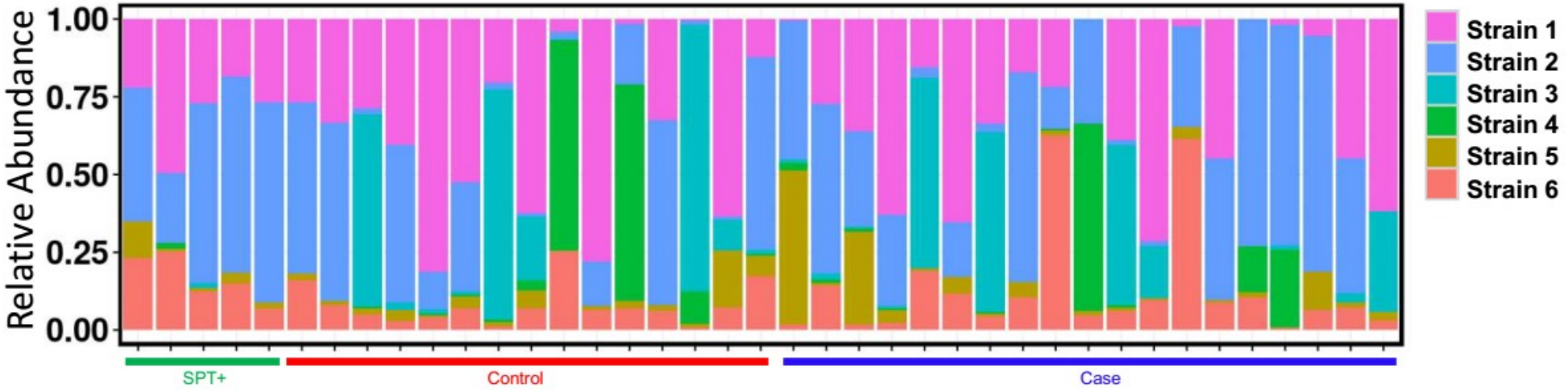
SPT+. 5 HP with positive skin prick test.

At everyone two smears (from right and left antecubital fossae) by tape stripping. Results of two WMS-tests were averaged.

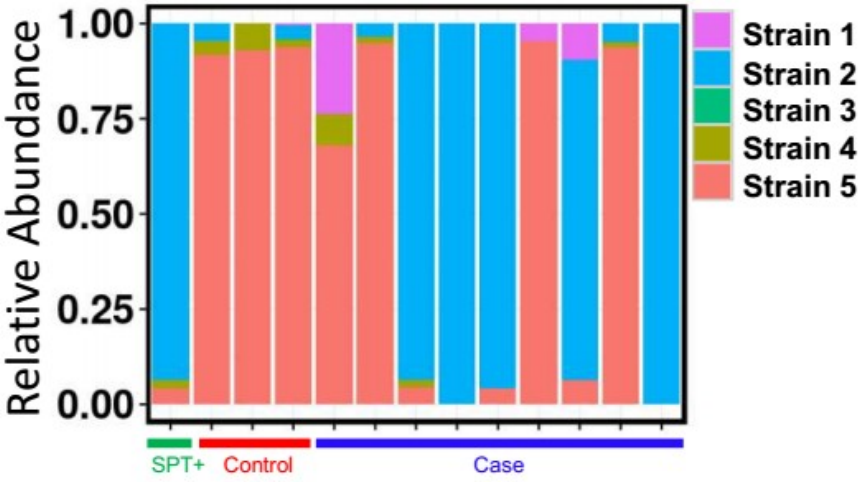
Chng KR, Tay AS, Li C. et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nat Microbiol. 2016 Jul 11;1(9):16106. 27562258.

Skin metagenome (15 HP SPT(-), 19 AtD+, 5 HP SPT+, WMS-test, Chng 2016). Representation of Staphylococcus aureus strains.

A



B



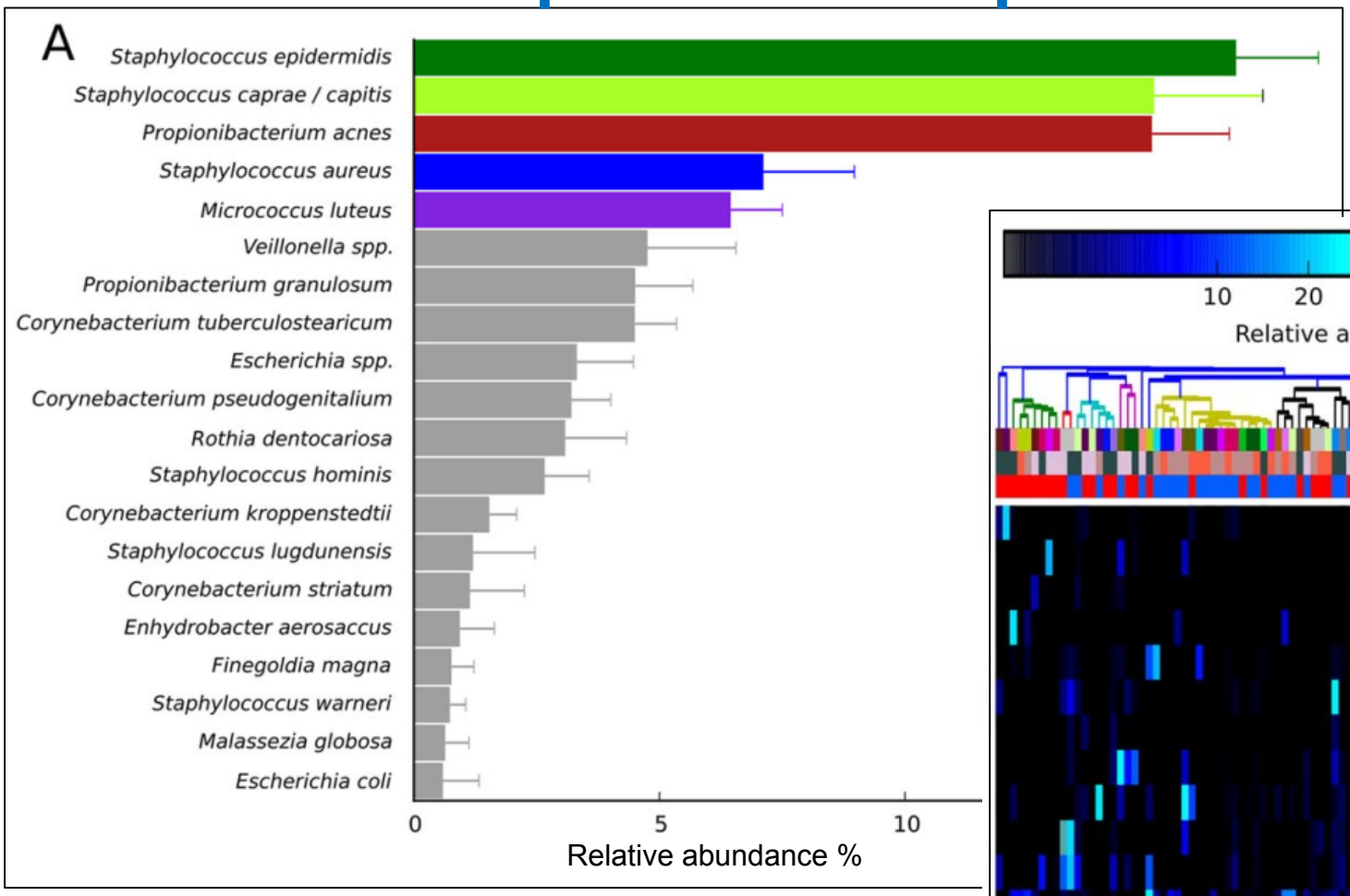
SPT+. 5 HP with positive skin prick test.

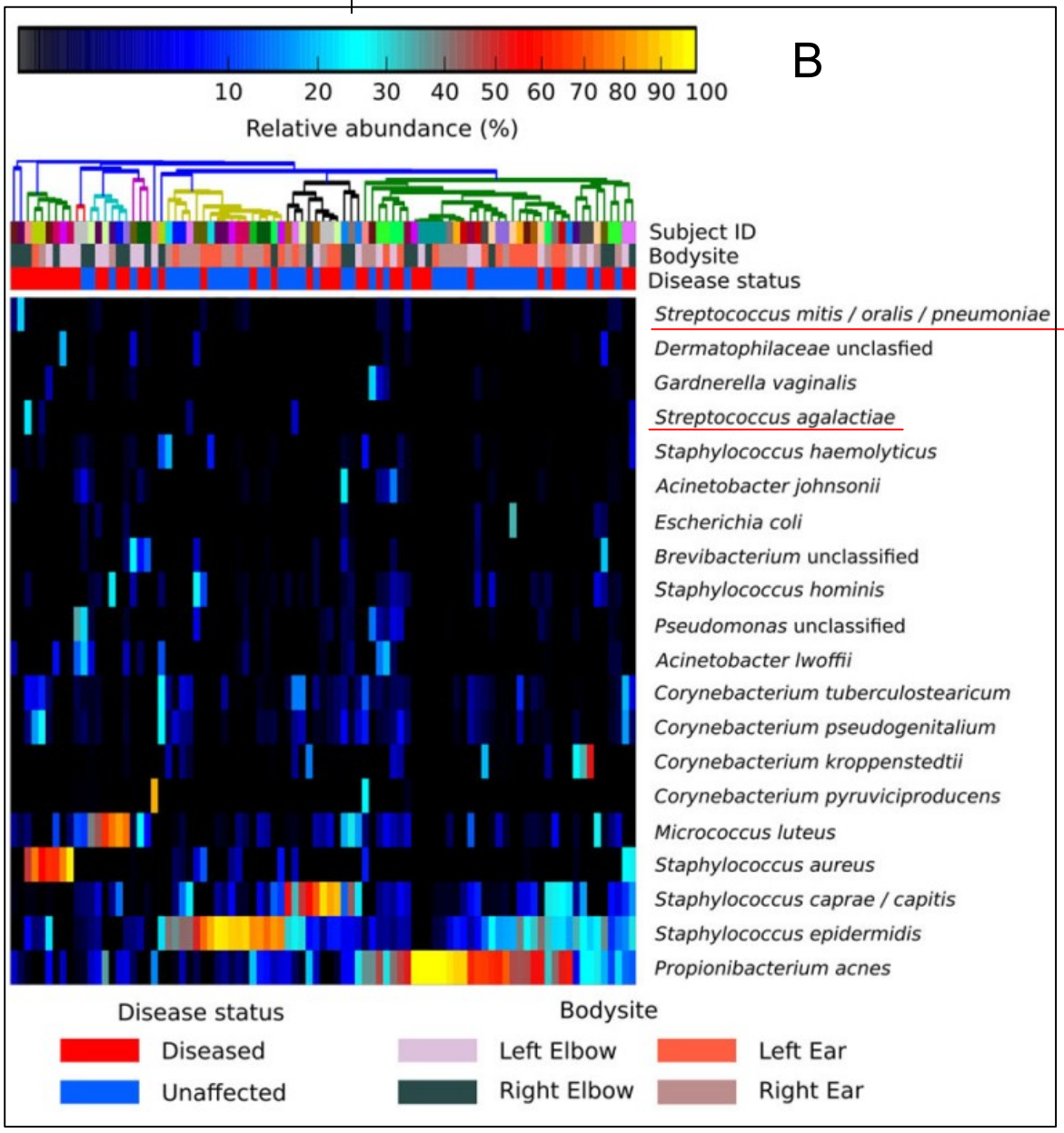
Control. 15 HP with negative skin prick test.

Case. 19 AtD+ (patients with history of atopic dermatitis). Healthy skin.

Chng KR, Tay AS, Li C. et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nat Microbiol. 2016 Jul 11;1(9):16106. 27562258.

Skin metagenome (28 PP, smears, WMS-test, Tett 2017). Representation of species.

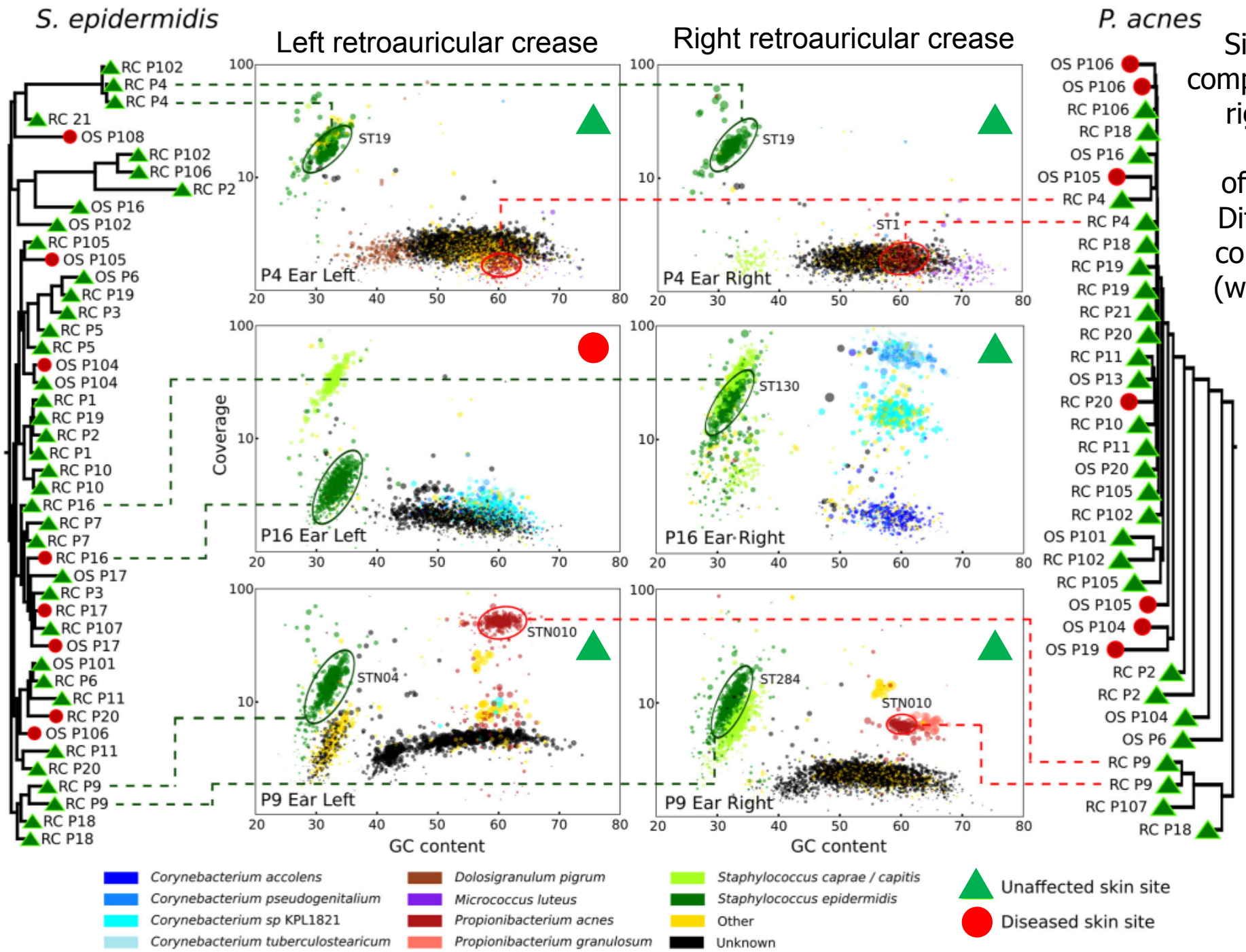




(A) Most presented species of retroauricular crease skin;
(B) Taxonomical structure of metagenomes (MetaPhlAn 2) for 20 most presented bacteria species.
It is grouped according to Bray-Curtis.

(A) – Fig.3a; (B) – Fig.1a from Tett A, Pasolli E, Farina S. et al. Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. NPJ Biofilms Microbiomes. 2017 Jun 22;3:14. 28649415.

Skin metagenome (28 PP, smears, WMS-test, Tett 2017). Similarity and difference of strains composition.



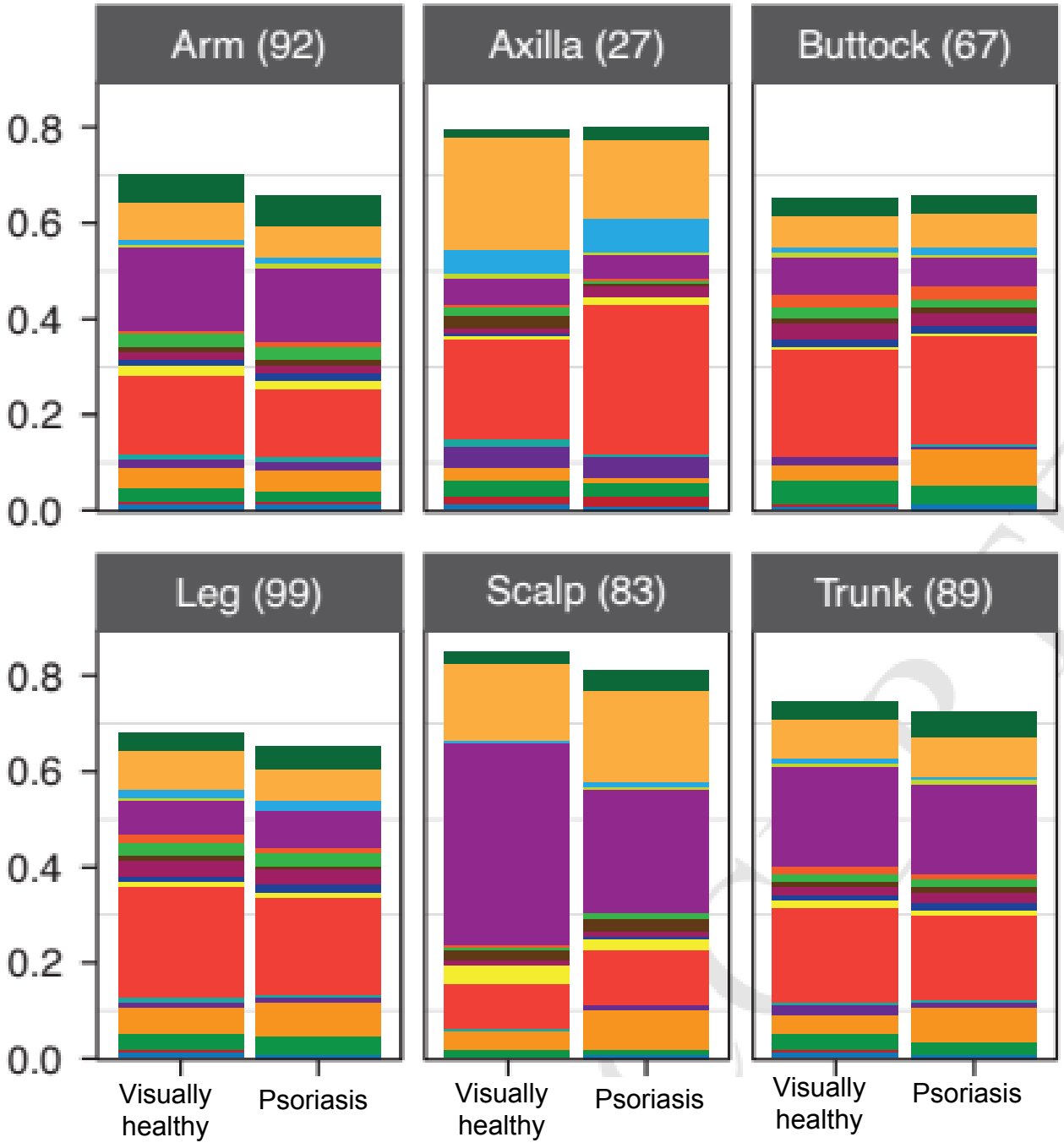
Similarity in strain composition for left and right ears without psoriasis of PP (P4 and P9). Difference in strain composition for left (with psoriasis) and right (without psoriasis) ear of PP (P16).

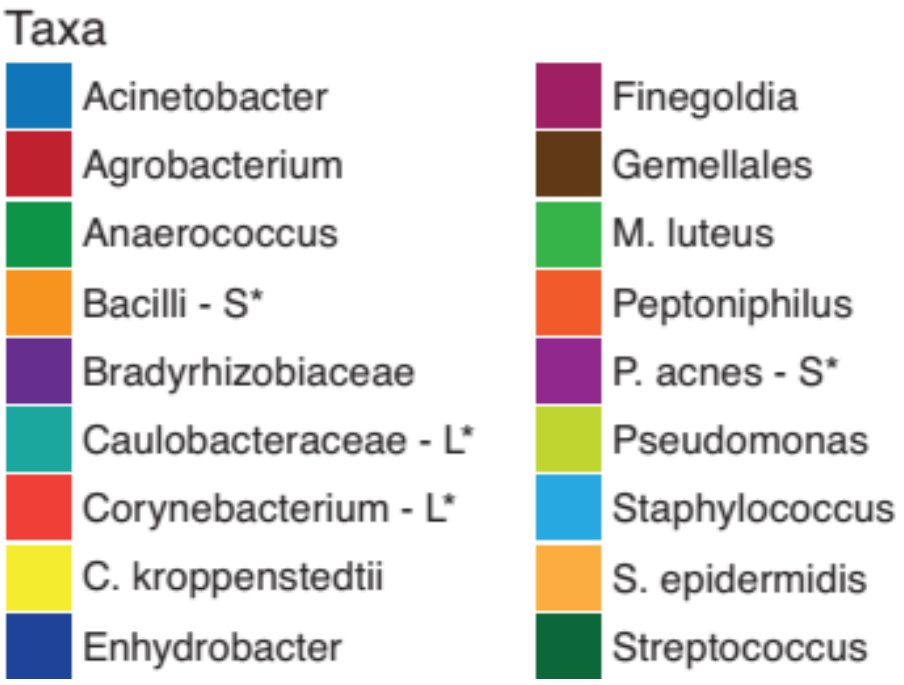
axis X - % GC in strain genome, axis Y – coverage

(Software of StrainPhlAn is applied to classification)

Fig.4 from Tett A, Pasolli E, Farina S. et al. Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. NPJ Biofilms Microbiomes. 2017 Jun 22;3:14. 28649415.

Skin metagenome (114 PP, smears, 16S-test, Loesche 2018). Composition and representation of main taxons.





Structure and proportions of main taxons on psoriatic and visually healthy skin are similar, but depend on localization. The number of samples for which averaging was performed is given in brackets. 14 out of 18 taxons with representation of > 1% in all samples are enumerated.

Fig.1 from Loesche MA, Farahi K, Capone K. et al. Longitudinal Study of the Psoriasis-Associated Skin Microbiome during Therapy with Ustekinumab in a Randomized Phase 3b Clinical Trial. 2018 Sep;138(9):1973-1981. 29559344.

Metagenomes of blood and psoriatic skin. Research project.

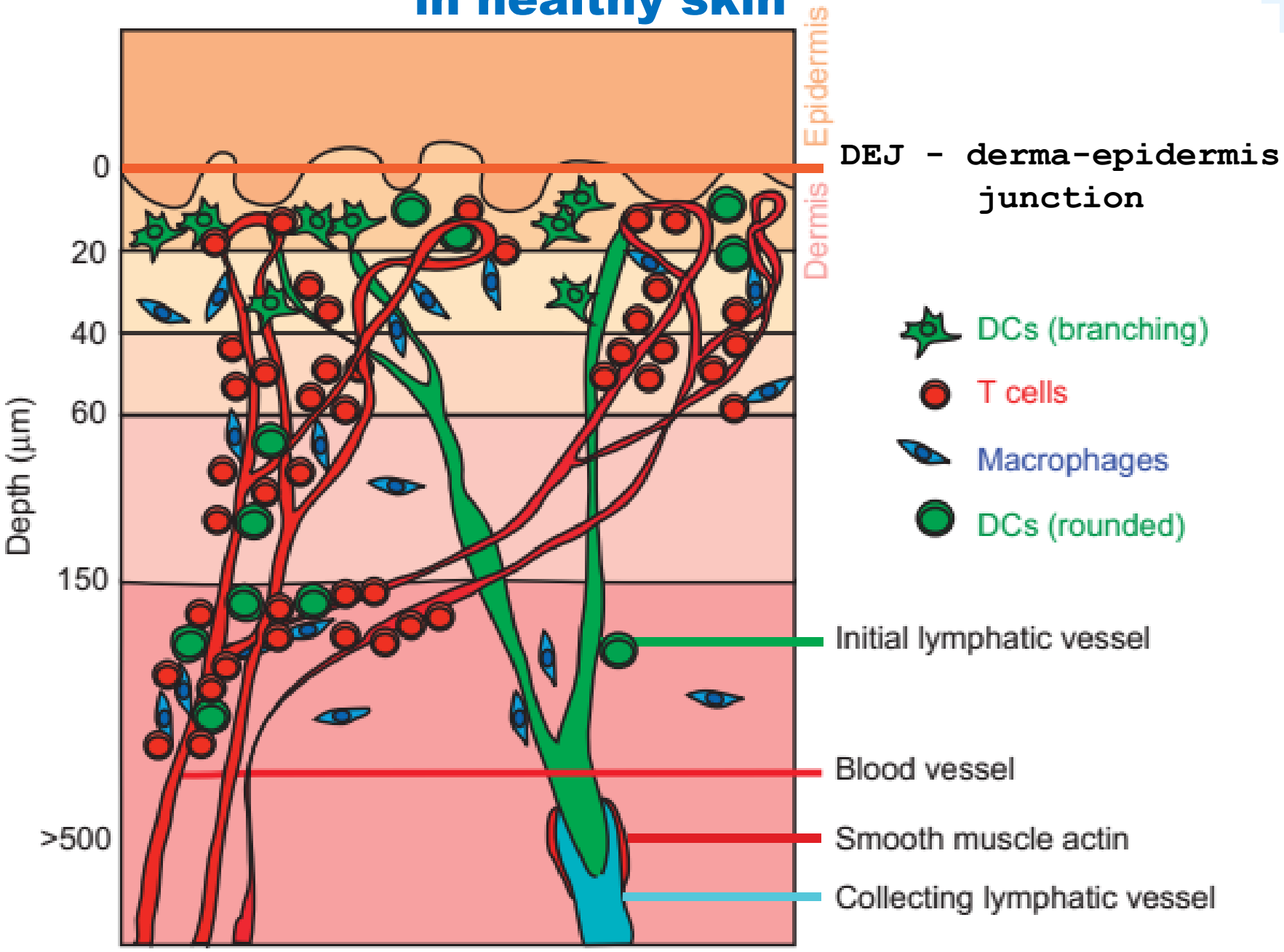
Section 3.

Phagocytes of normal and psoriatic skin.

NET - neutrophil extracellular traps in blood and in psoriatic skin.

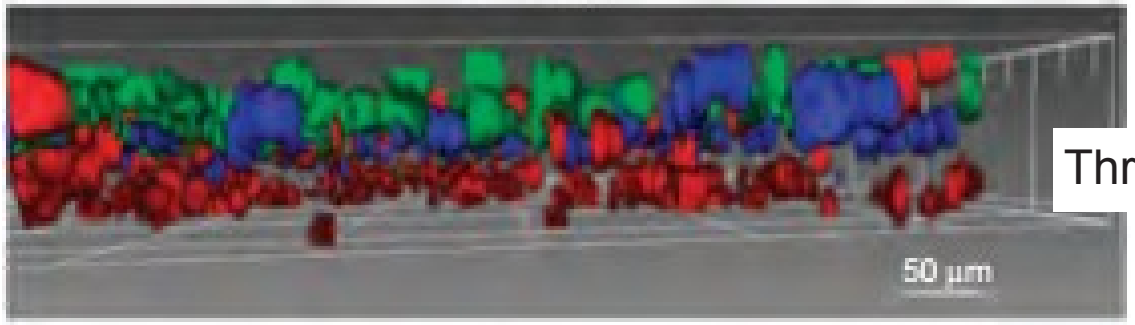
New models of psoriasis pathogenesis.

Dendritic cells, macrophages and T-lymphocytes in healthy skin



Fragment of Fig.4 from Wang XN, McGovern N, Gunawan M. et al. A Three-Dimensional Atlas of Human Dermal Leukocytes, Lymphatics, and Blood Vessels. J Invest Dermatol. 2014 Apr;134(4):965-74. 24352044.

Dendritic cells, macrophages and T-lymphocytes in healthy skin (3D)



Three types of cells together

3D - distribution under DEJ to 0 to 60 microns' depth

CD11c+ DC – dendritic cells

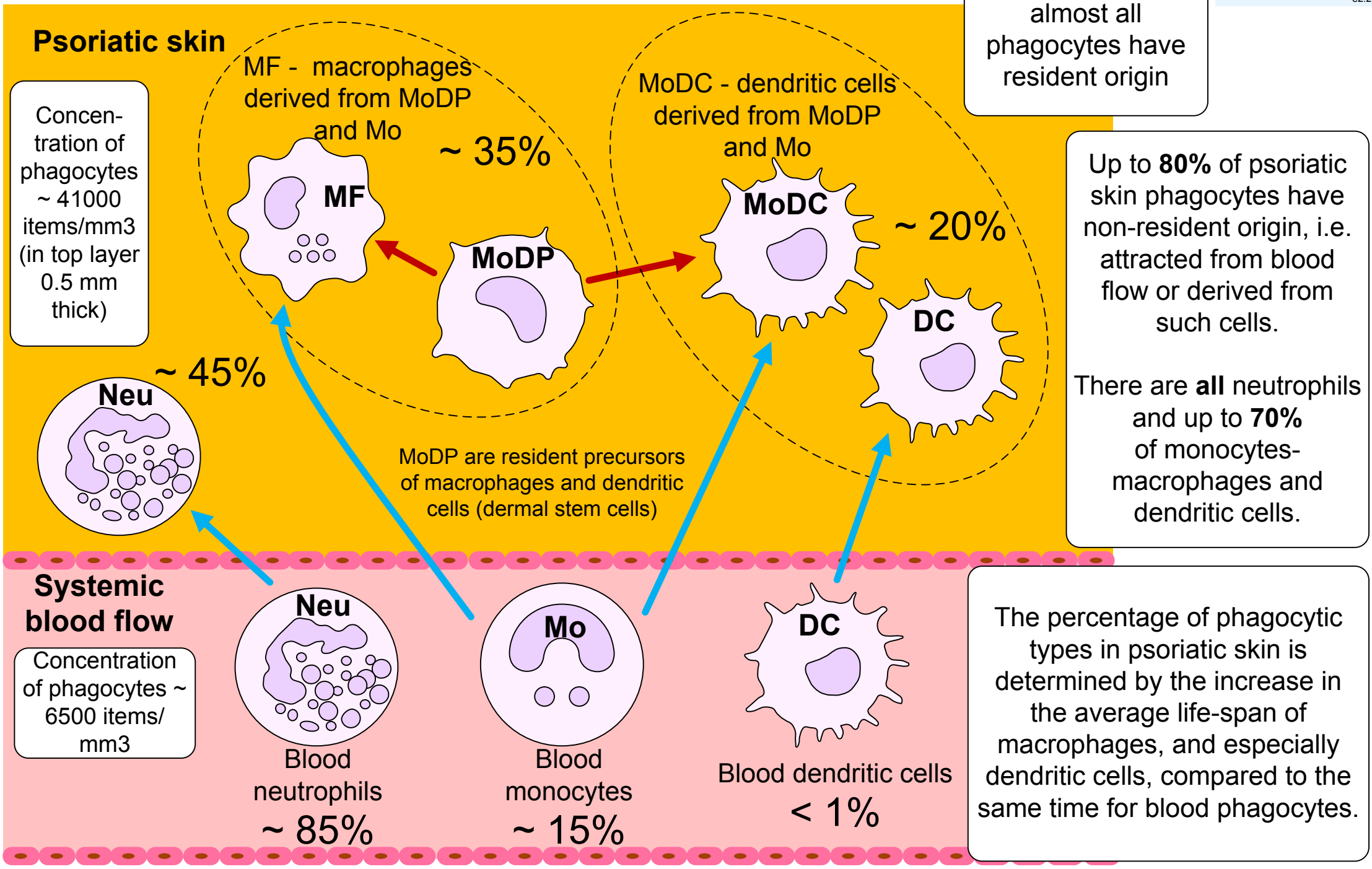
FXIIIa+ MF - macrophages

CD3+ T-lymphocytes

Fragment of Fig.3 from Wang XN, McGovern N, Gunawan M. et al. A Three-Dimensional Atlas of Human Dermal Leukocytes, Lymphatics, and Blood Vessels. J Invest Dermatol. 2014 Apr;134(4):965-74. 24352044.

Attraction of blood phagocytes in skin at moderate-severe psoriasis

In healthy skin almost all phagocytes have resident origin

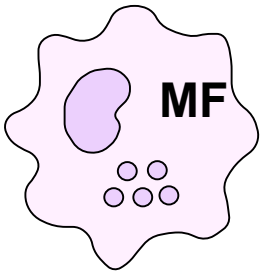


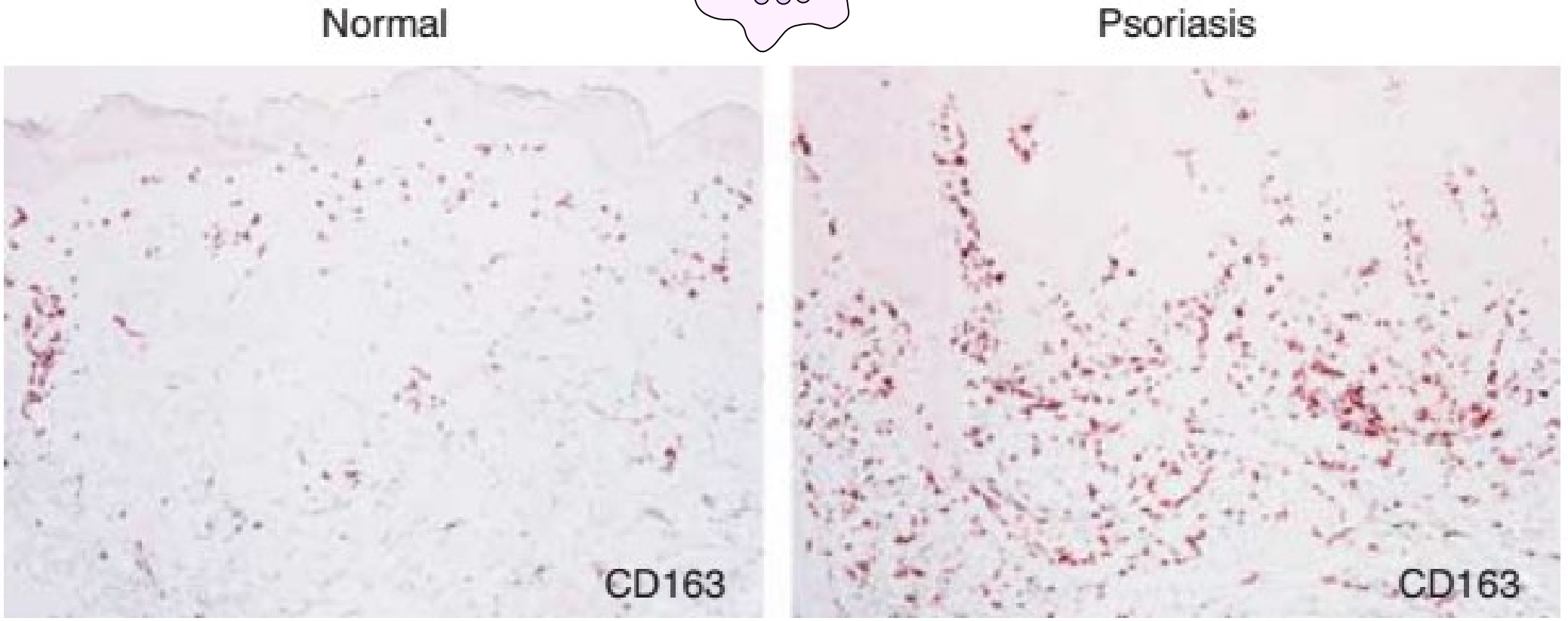
Up to **80%** of psoriatic skin phagocytes have non-resident origin, i.e. attracted from blood flow or derived from such cells.

There are **all** neutrophils and up to **70%** of monocytes-macrophages and dendritic cells.

The percentage of phagocytic types in psoriatic skin is determined by the increase in the average life-span of macrophages, and especially dendritic cells, compared to the same time for blood phagocytes.

Macrophages in healthy and psoriatic skin





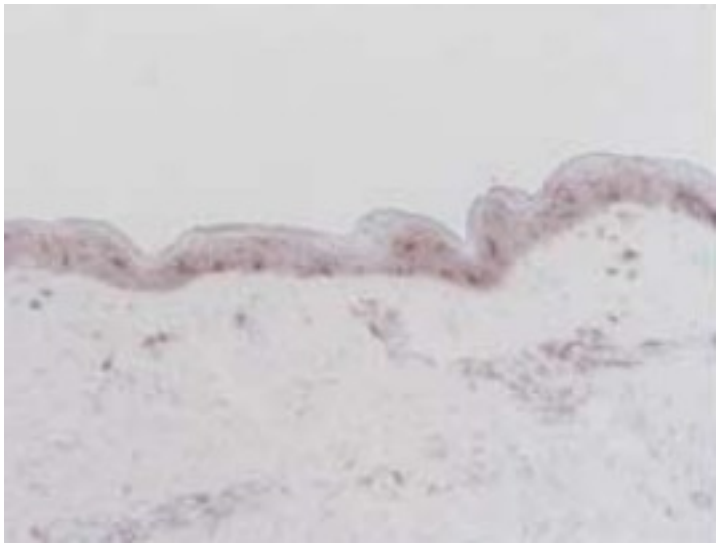
CD163+ macrophages.

Fragment of Fig. 1 from Fuentes-Duculan J, Suárez-Fariñas M, Zaba LC et al. A Subpopulation of CD163-Positive Macrophages Is Classically Activated in Psoriasis. Journal of Investigative Dermatology 2010 Oct; 130:2412-2422. 20555352.

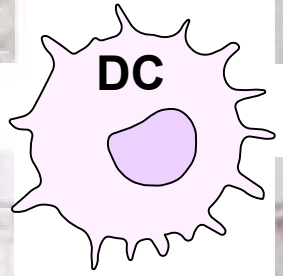
Dendritic cells in healthy and psoriatic skin

Norm

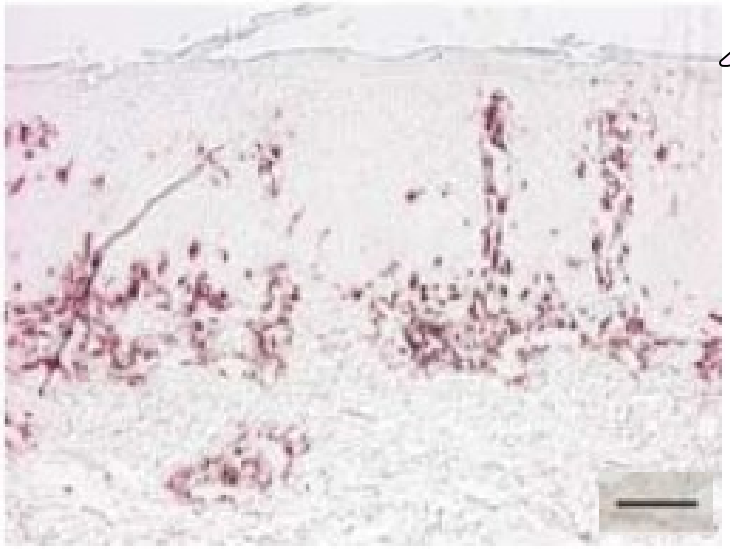
CD11c+

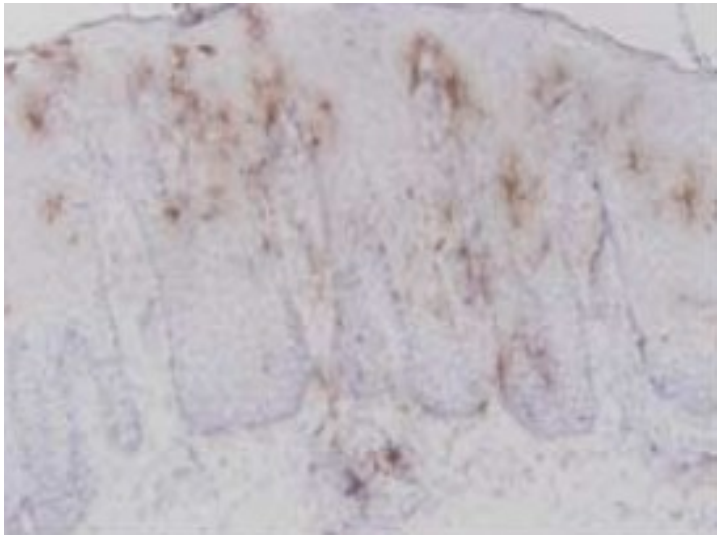


CD1a+



Psoriasis

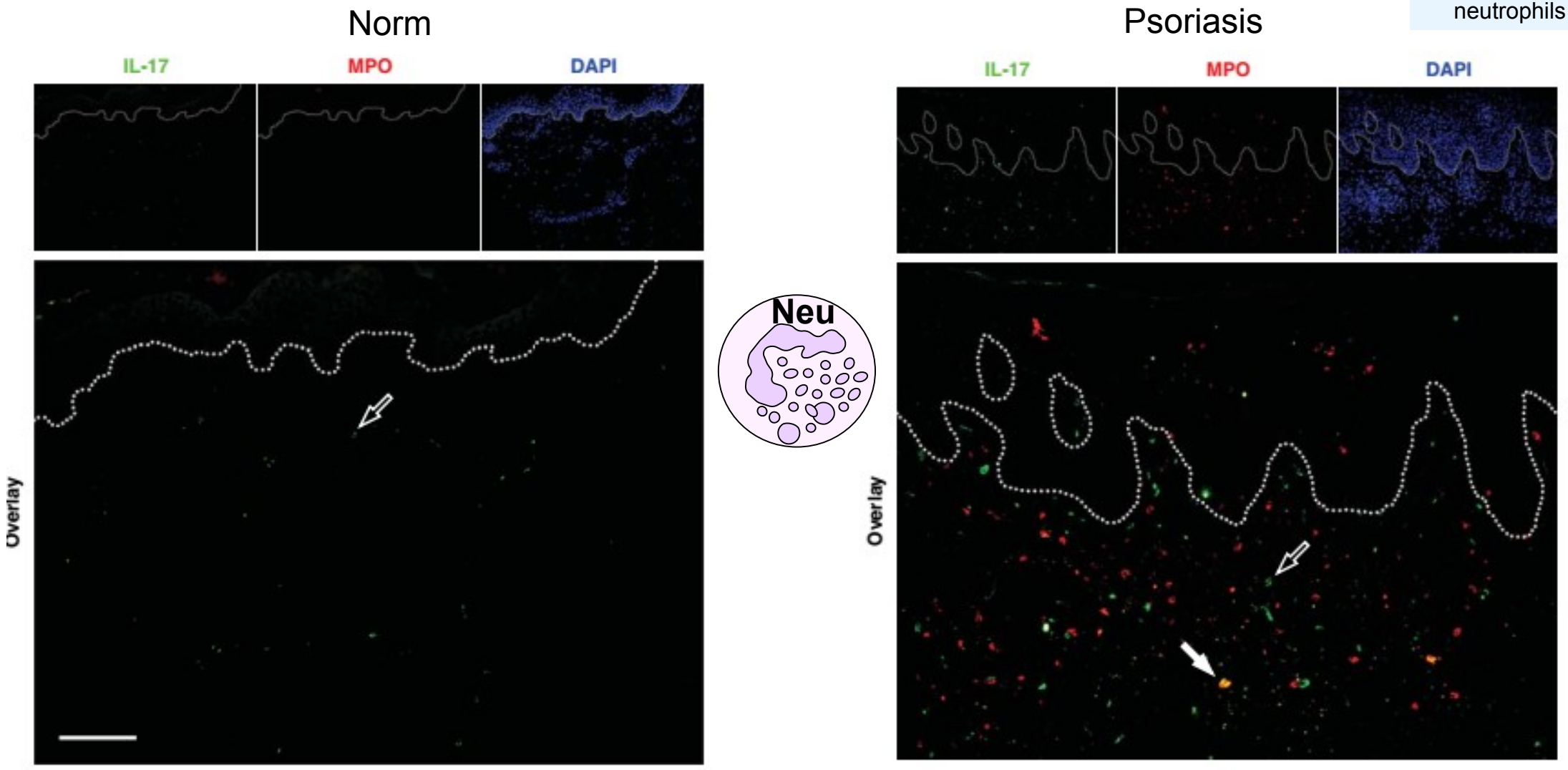




Fragment of Fig.1 from Zaba LC, Fuentes-Duculan J, Eungdamrong NJ et al. Psoriasis Is Characterized by Accumulation of Immunostimulatory and Th1/Th17 Cell-Polarizing Myeloid Dendritic Cells. J Invest Dermatol. 2009 Jan;129(1):79-88. 18633443.

Fragment of Fig.2 from Komine M, Karakawa M, Takekoshi T. et al. Early inflammatory changes in the "perilesional skin" of psoriatic plaques: is there interaction between dendritic cells and keratinocytes? J Invest Dermatol. 2007 Aug;127(8):1915-22. 17446902.

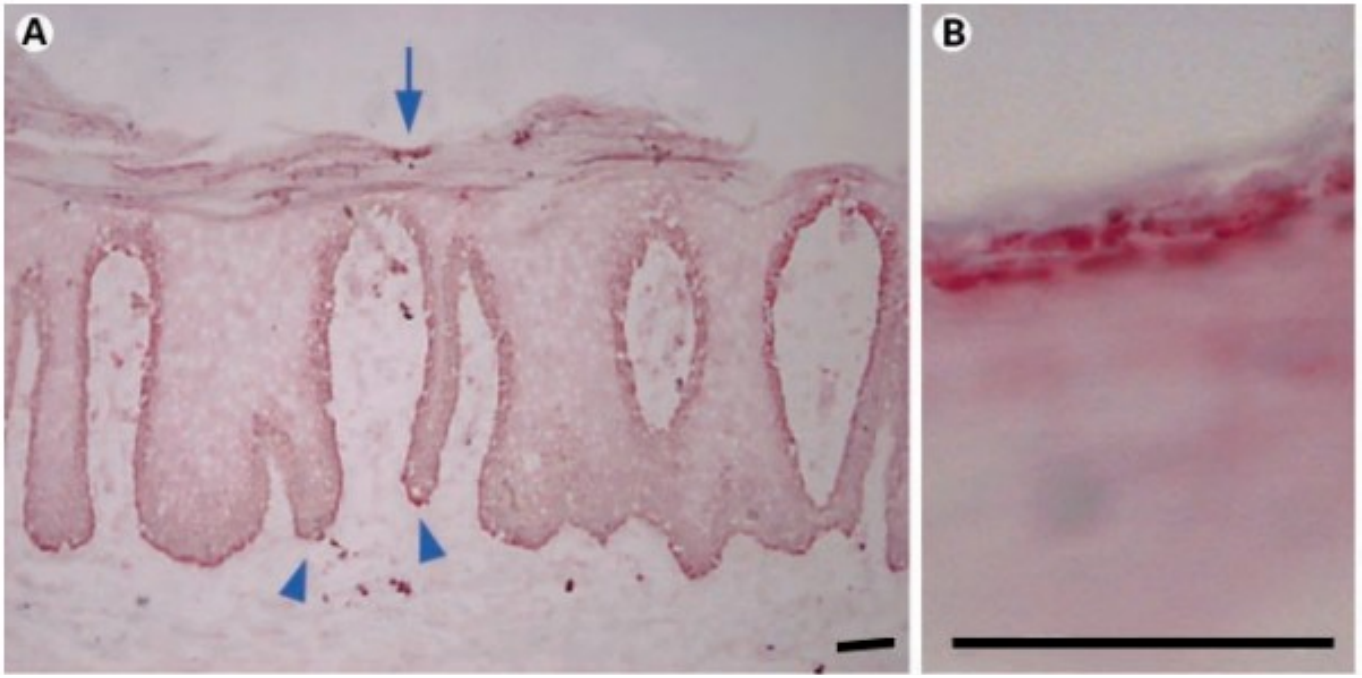
Neutrophils in healthy and psoriatic skin

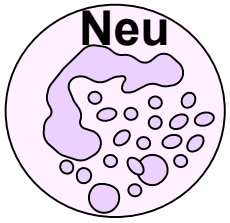


Neutrophils (red) containing IL-17 (green) are observed in psoriatic plaque. Immunofluorescence is performed for IL-17 (green), MPO - myeloperoxidase (red) and DAPI (blue). Characteristic image, one of 12 for HP (left) and one of 12 PP (right). 200x zoom. Dashed line denotes derma-epidermis junction. Scale bar (below at left) = 100 microns.

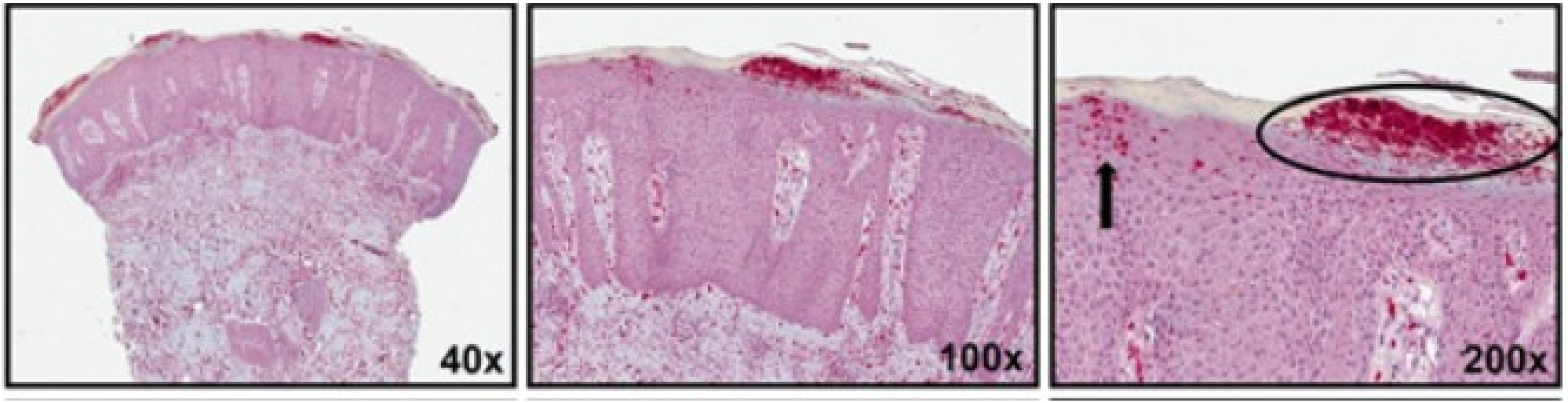
Fragment of Fig. 4 from Annex to Lin AM, Rubin CJ, Khandpur R. et al. Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis. J Immunol.2011 Jul 1;187(1):490-500. 21606249.

Neutrophils in psoriatic epidermis (Munro's abscesses)





Fragment of Fig.1 from Ozawa M, Terui T, Tagami H. Localization of IL-8 and complement components in lesional skin of psoriasis vulgaris and pustulosis palmaris et plantaris. Dermatology. 2005;211(3):249-55. 16205070.



Fragment of Fig.3 from Reich K, Papp KA, Matheson RT. et al. Evidence that a neutrophil-keratinocyte crosstalk is an early target of IL-17A inhibition in psoriasis. Exp Dermatol. 2015 Jul;24(7):529-35. 25828362.

Netosis - formation of NET (neutrophil extracellular traps)

Triggers of NET formation from neutrophils

LPS, FMLP, PMA, M1 protein with fibrinogen, thrombocytes and LPS, cytokines (IL-1beta, IL-8, IL-18), bacteria, fungi, viruses, parasites, autoantibody.

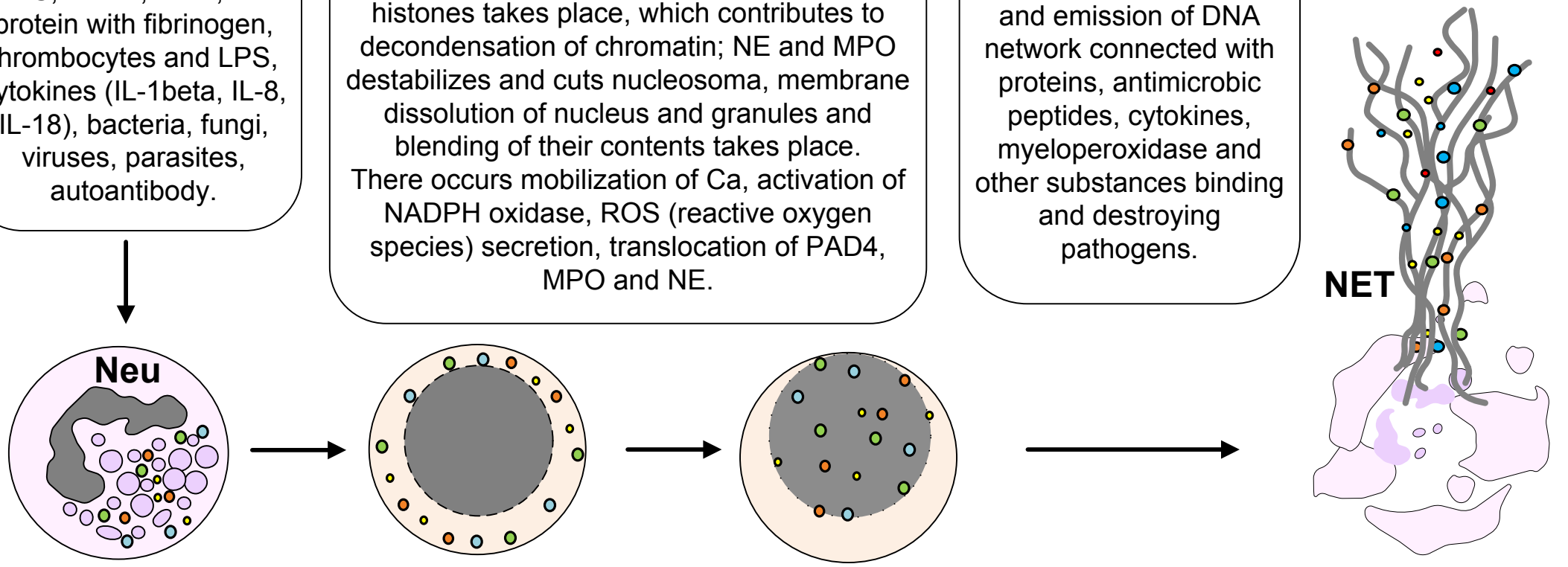
Internal neutrophil transformations preceding NET formation

With PAD4 participation, citrullination of histones takes place, which contributes to decondensation of chromatin; NE and MPO destabilizes and cuts nucleosoma, membrane dissolution of nucleus and granules and blending of their contents takes place. There occurs mobilization of Ca, activation of NADPH oxidase, ROS (reactive oxygen species) secretion, translocation of PAD4, MPO and NE.

Explosive NET formation

Rupture of cellular wall and emission of DNA network connected with proteins, antimicrobic peptides, cytokines, myeloperoxidase and other substances binding and destroying pathogens.

NET size can 10-15 times exceed neutrophil size.



In 2004, netosis was discovered – a new mechanism which lets neutrophils perform protective functions by forming extracellular network. Netosis is a type of cellular death of neutrophils, on an equal basis with apoptosis and necrosis.

NET composition

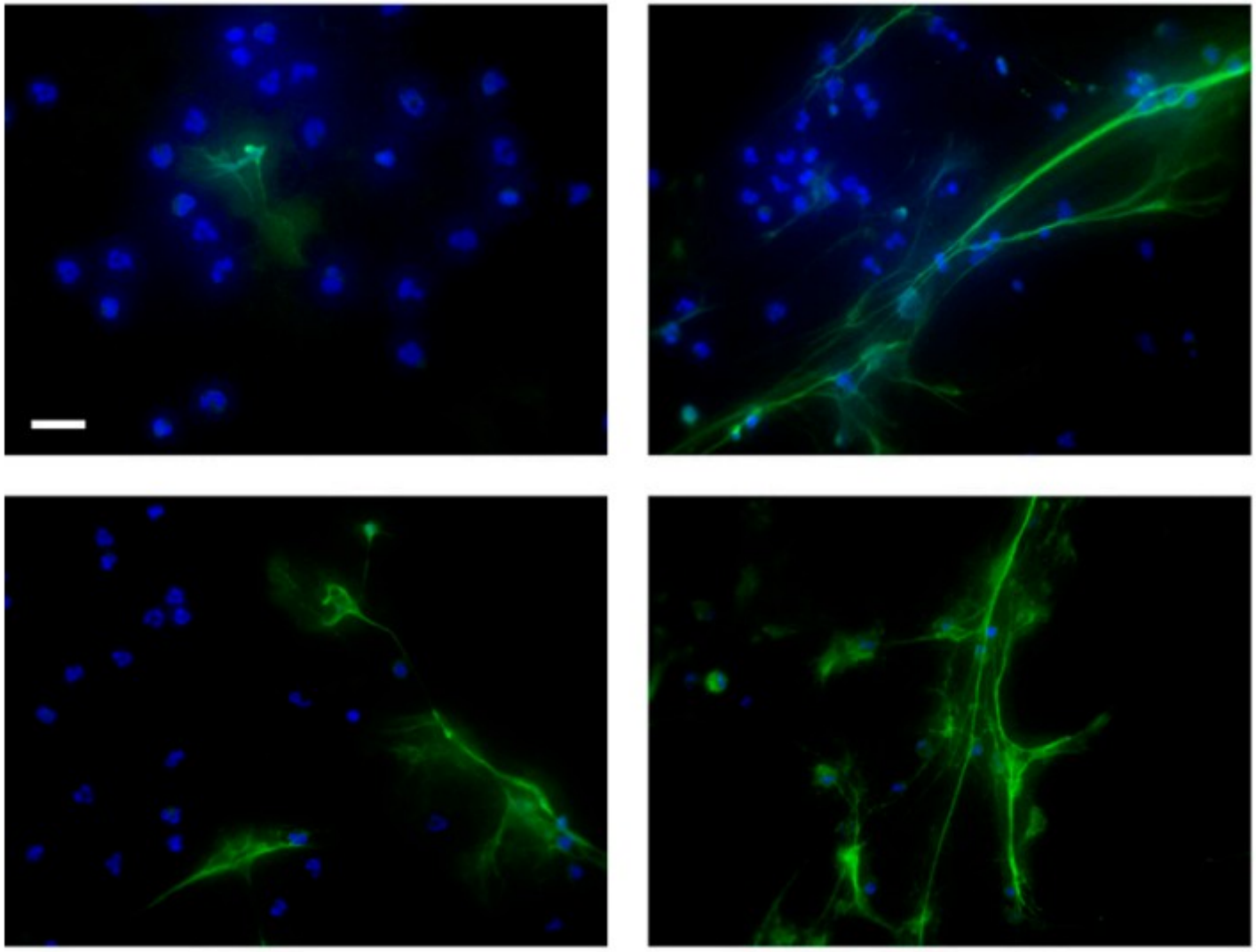
hDNA (host DNA), MPO (myeloperoxidase), NE (neutrophil elastase), cathepsin G, citrullinated histones, antimicrobic proteins, LL37, cytokines (IL-17 and other).
In total more than 30 various proteins.

Based on Fig.1 from Hasler P, Giaglis S, Hahn S. Neutrophil extracellular traps in health and disease. Swiss Med Wkly. 2016 Oct 10;146:w14352. PMID 27723901.

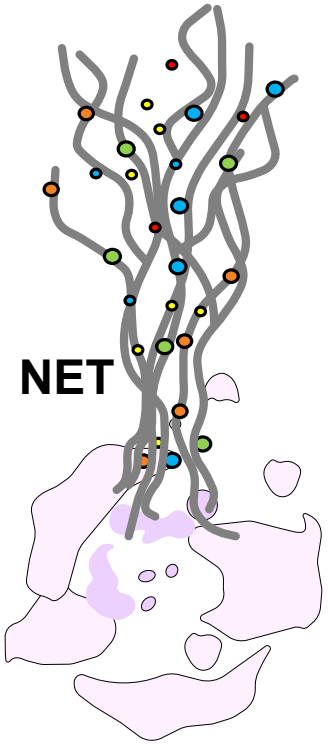
NET in healthy and psoriatic blood (Lin 2011)

Norm

Psoriasis



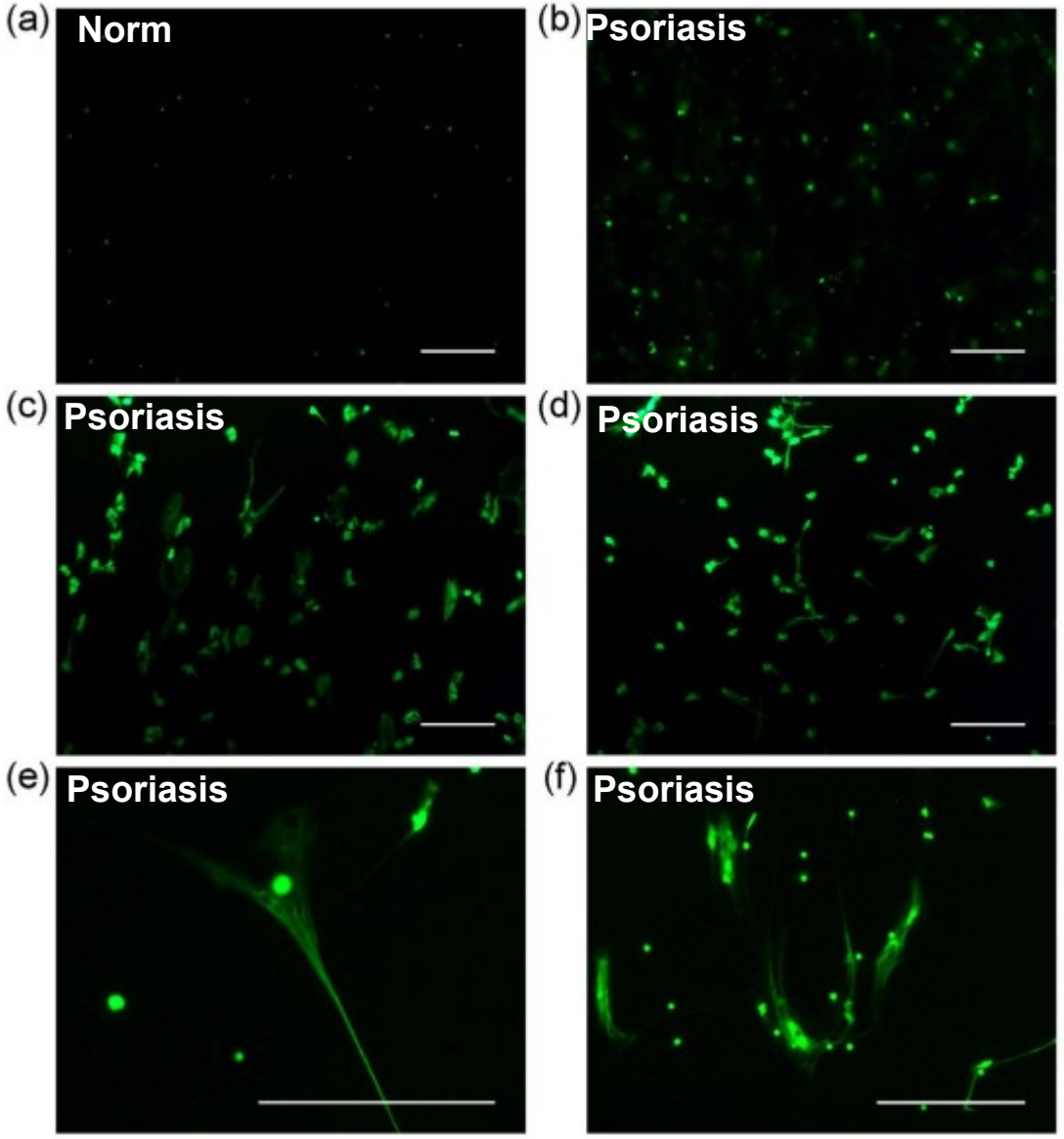
At once (in 0 hours) In 2 hours

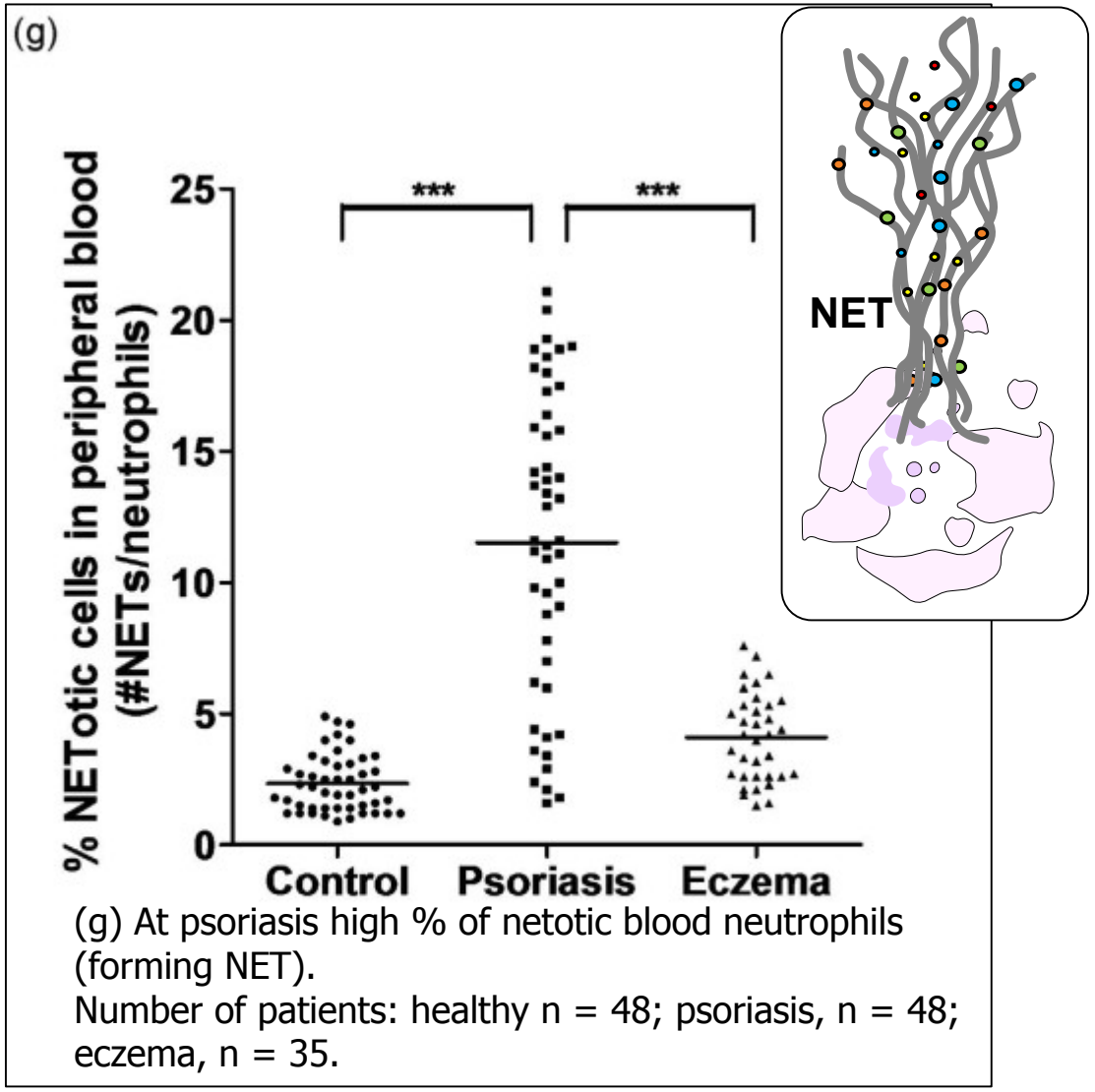


Neutrophils (blue) in healthy and psoriatic blood (in vitro) undergo netosis (at once and in 2 hours). 400 x increase, Immunofluorescence it is executed for Hoechst 33342 (blue) and neutrophil elastase (green). Scale bar = 20 microns.

Fragment of Fig.4 from Lin AM, Rubin CJ, Khandpur R. et al. Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis. J Immunol.2011 Jul 1;187(1):490-500. PMID 21606249.

NET in healthy and psoriatic blood (Hu 2016)



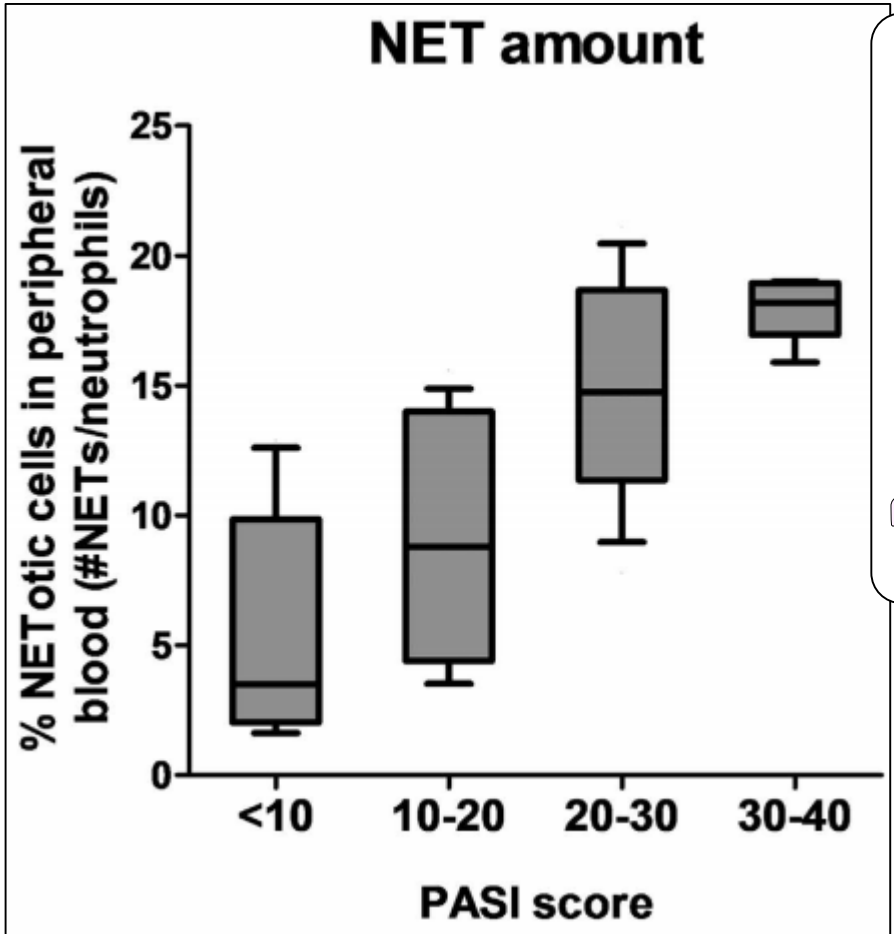


(g) At psoriasis high % of netotic blood neutrophils (forming NET).
Number of patients: healthy n = 48; psoriasis, n = 48; eczema, n = 35.

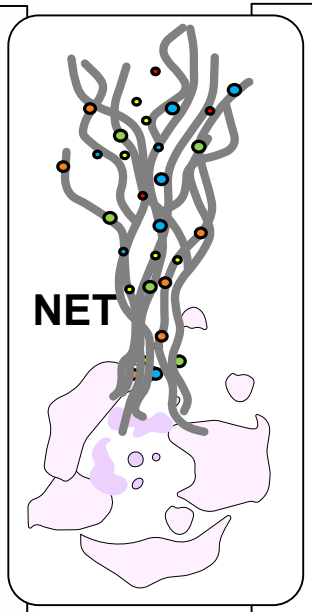
The number of the netotic neutrophils (forming NET) in blood are low at norm (a) and high at psoriasis (b-d). During netosis there is expansion of nucleus and formation of extracellular network hDNA (e, f).
Extracellular hDNA is painted by fluorescent Sytox Green. Scale bar = 200 microns.

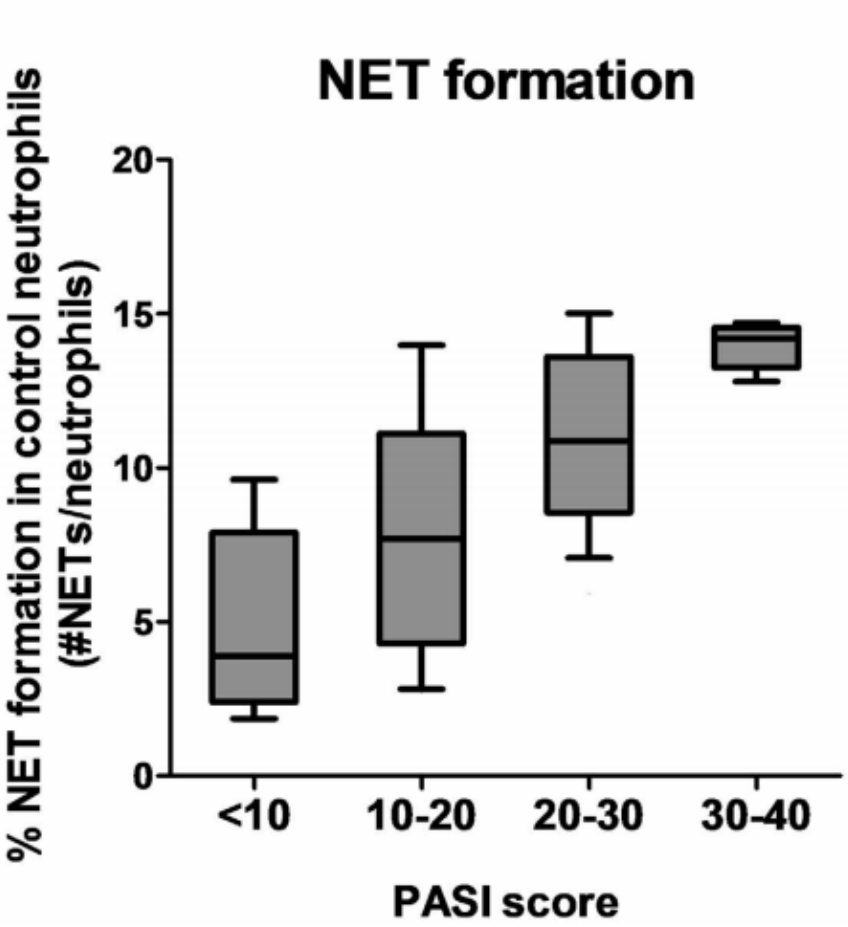
Fig.1 from Hu SC, Yu HS, Yen FL. et al. Neutrophil extracellular trap formation is increased in psoriasis and induces human β-defensin-2 production in epidermal keratinocytes. Sci Rep. 2016 Aug 5;6:31119, PMID 27493143.

Correlation between PASI and percentage of netotic blood neutrophils (Hu 2016)



(a) Correlation between PASI and percentage of netotic neutrophils (forming NET) in blood at psoriasis.



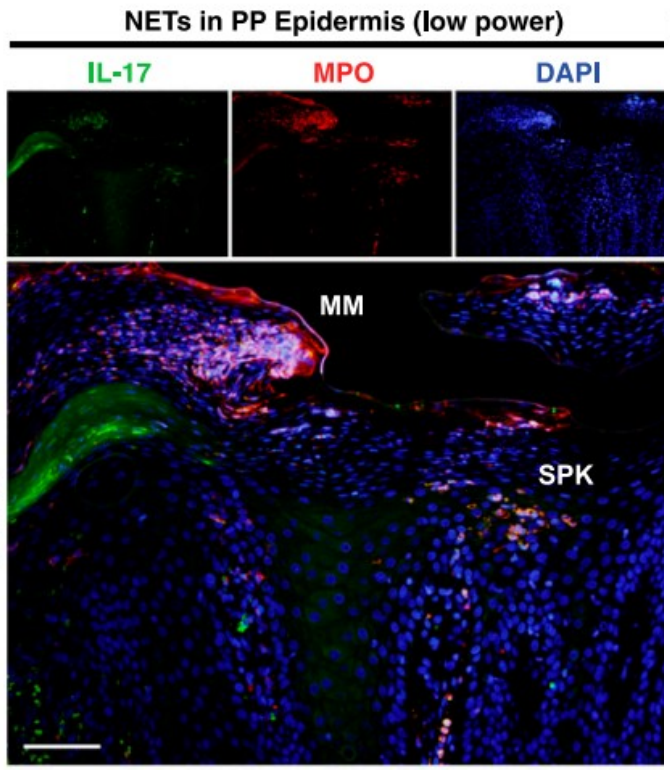


(b) Correlation between percentage of netotic neutrophils (forming NET) in control healthy blood under the influence of serum of psoriatic patients with various PASI.

PASI < 10, n=10; PASI 10-20, n=15; PASI 20-30, n=18; PASI 30-40, n=5.

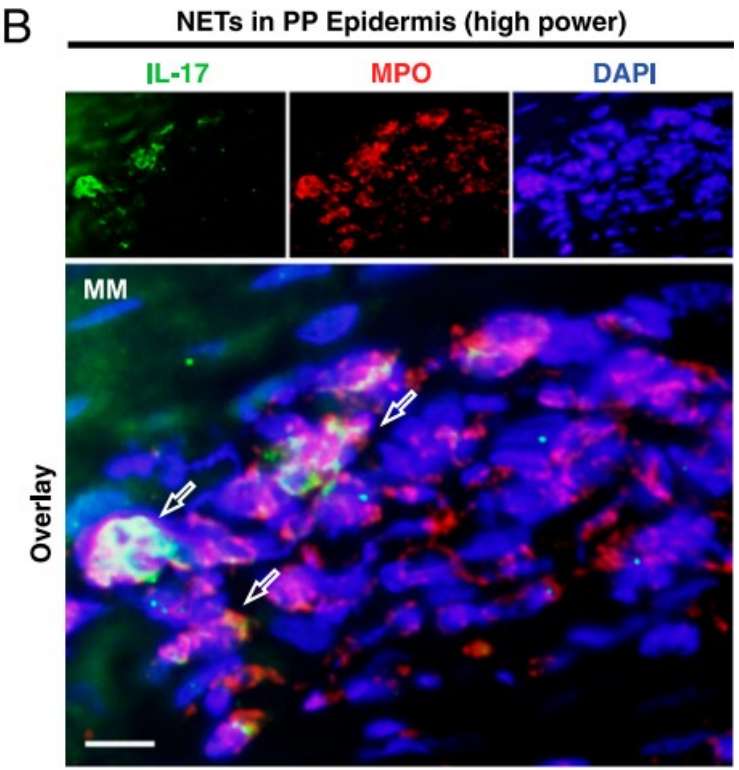
Fig.3 from Hu SC, Yu HS, Yen FL. et al. Neutrophil extracellular trap formation is increased in psoriasis and induces human β-defensin-2 production in epidermal keratinocytes. Sci Rep. 2016 Aug 5;6:31119, PMID 27493143.

NET in psoriatic skin (Lin 2011)

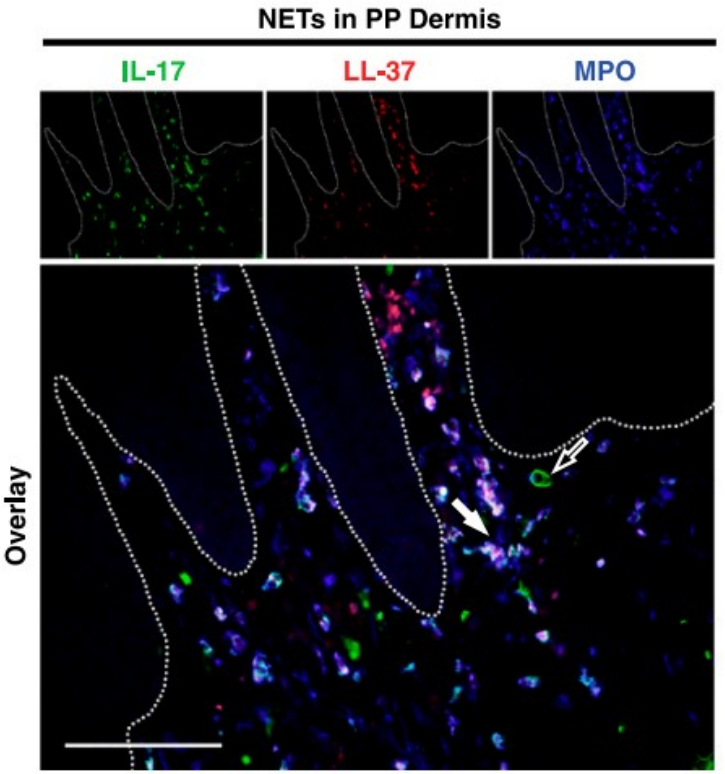


A. Psoriatic epidermis contains IL-17+ neutrophils in Munro microabscesses (MM) and in spongiform pustules of Kogoj (SPK).
200 x zoom.
Scale bar = 100 microns.

B



B. Munro Microabscess (MM). NET formation is accompanied by emission of nuclear material (hollow arrows) and contains MPO and IL-17.
1000 x zoom.
Scale bar = 10 microns.

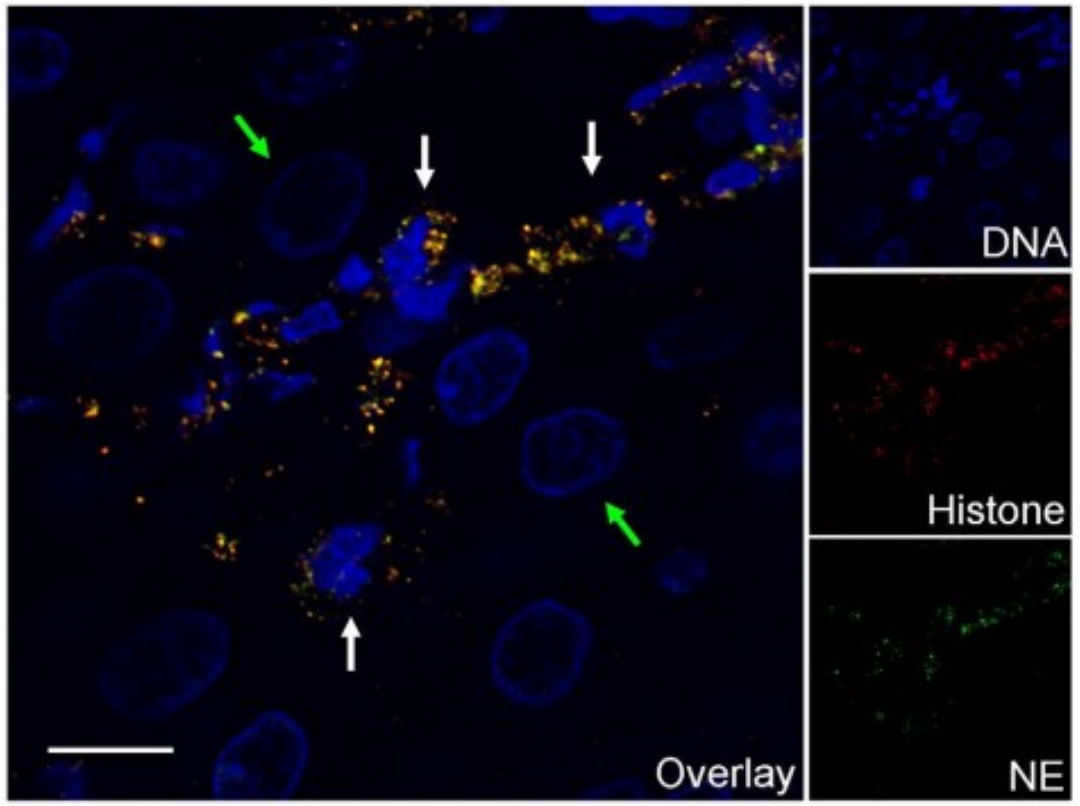


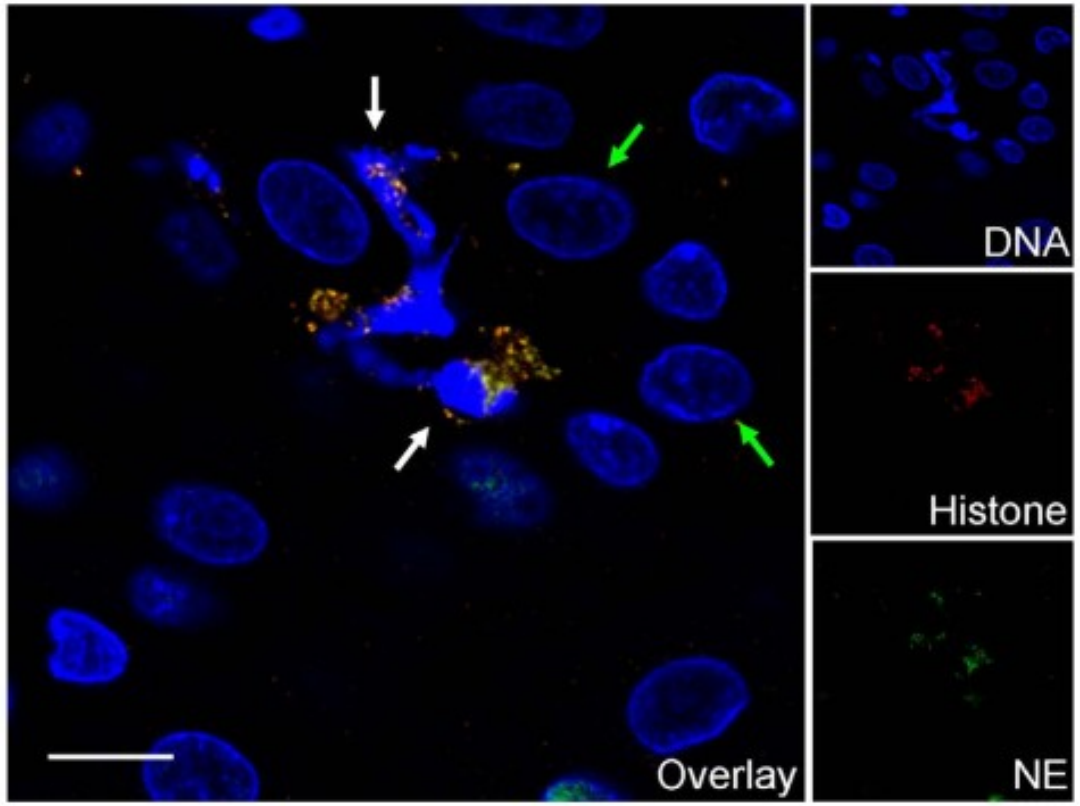
C. Biopsy of psoriatic skin (n = 3). Immunofluorescence for IL-17 (green), LL-37 (red), and MPO (blue). The number of dermal NET (white color, continuous arrow) as well as IL-17+ cells, other than neutrophils (green color, hollow arrow) were discovered.
Scale bar = 100 microns.

Biopsy of psoriatic epidermis (n = 12).
Immunofluorescence for IL-17 (green), MPO - myeloperoxidase (red) and DAPI (4', 6-diamidino-2-phenylindole - blue).

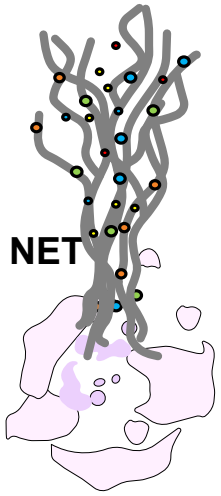
Fragment of Fig.3 from Lin AM, Rubin CJ, Khandpur R. et al. Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis. J Immunol.2011 Jul 1;187(1):490-500. PMID 21606249.

NET in psoriatic epidermis (Hu 2016)



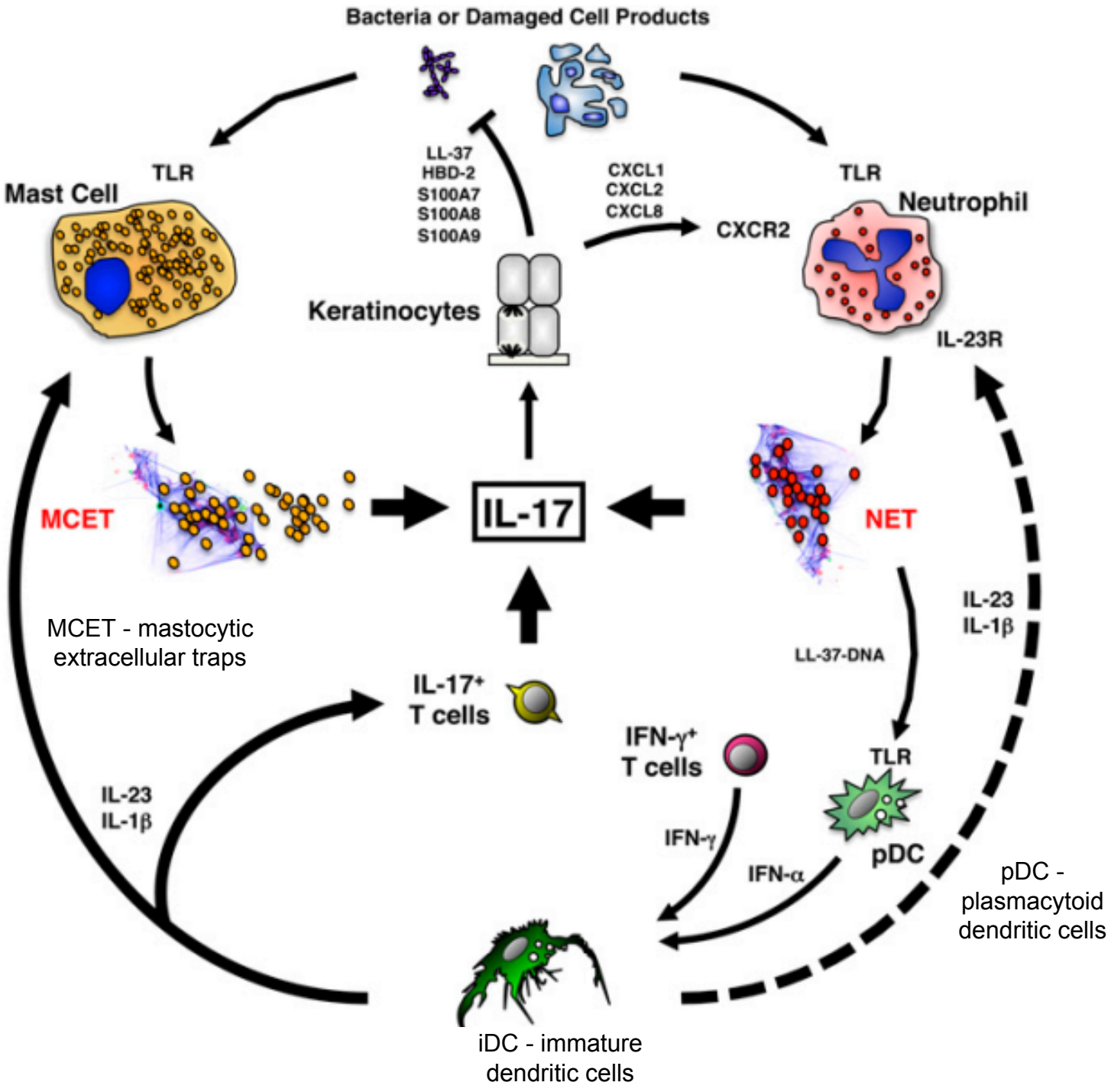


Confocal microscopy of psoriatic epidermis.
NET (white arrows) are often found near keratinocytes (green arrows). NET are identified by overlapping of images of extracellular DNA networks (DAPI, blue), histones (red) and neutrophil elastases (NE, green).
Scale bar = 10 microns.



Fragment of Fig..4 from Hu SC, Yu HS, Yen FL. et al. Neutrophil extracellular trap formation is increased in psoriasis and induces human β-defensin-2 production in epidermal keratinocytes. Sci Rep. 2016 Aug 5;6:31119, PMID 27493143.

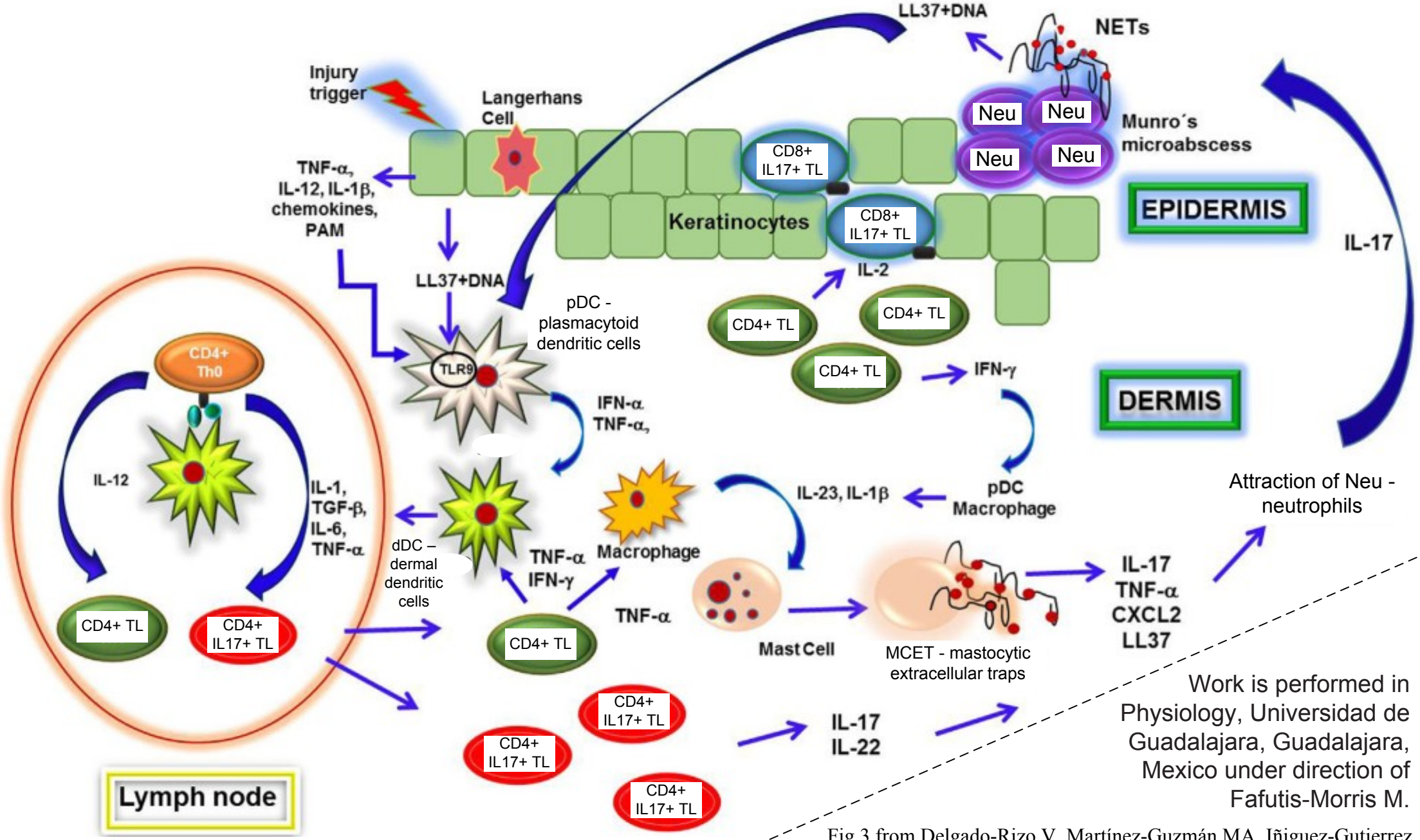
First scheme of psoriasis pathogenesis taking into account NET (Lin 2011)



The work was carried out at University of Michigan, Ann Arbor, MI, USA, supervised by Kaplan MJ and Bruce AT.

Fig.6 from Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, Villanueva EC, Shah P, Kaplan MJ, Bruce AT. Mast Cells and Neutrophils Release IL-17 through Extracellular Trap Formation in Psoriasis. J Immunol.2011 Jul 1;187(1):490-500. 21606249.

Model of psoriasis pathogenesis taking into account NET and MSET (Delgado-Rizo 2017)



Work is performed in Physiology, Universidad de Guadalajara, Guadalajara, Mexico under direction of Fafutis-Morris M.

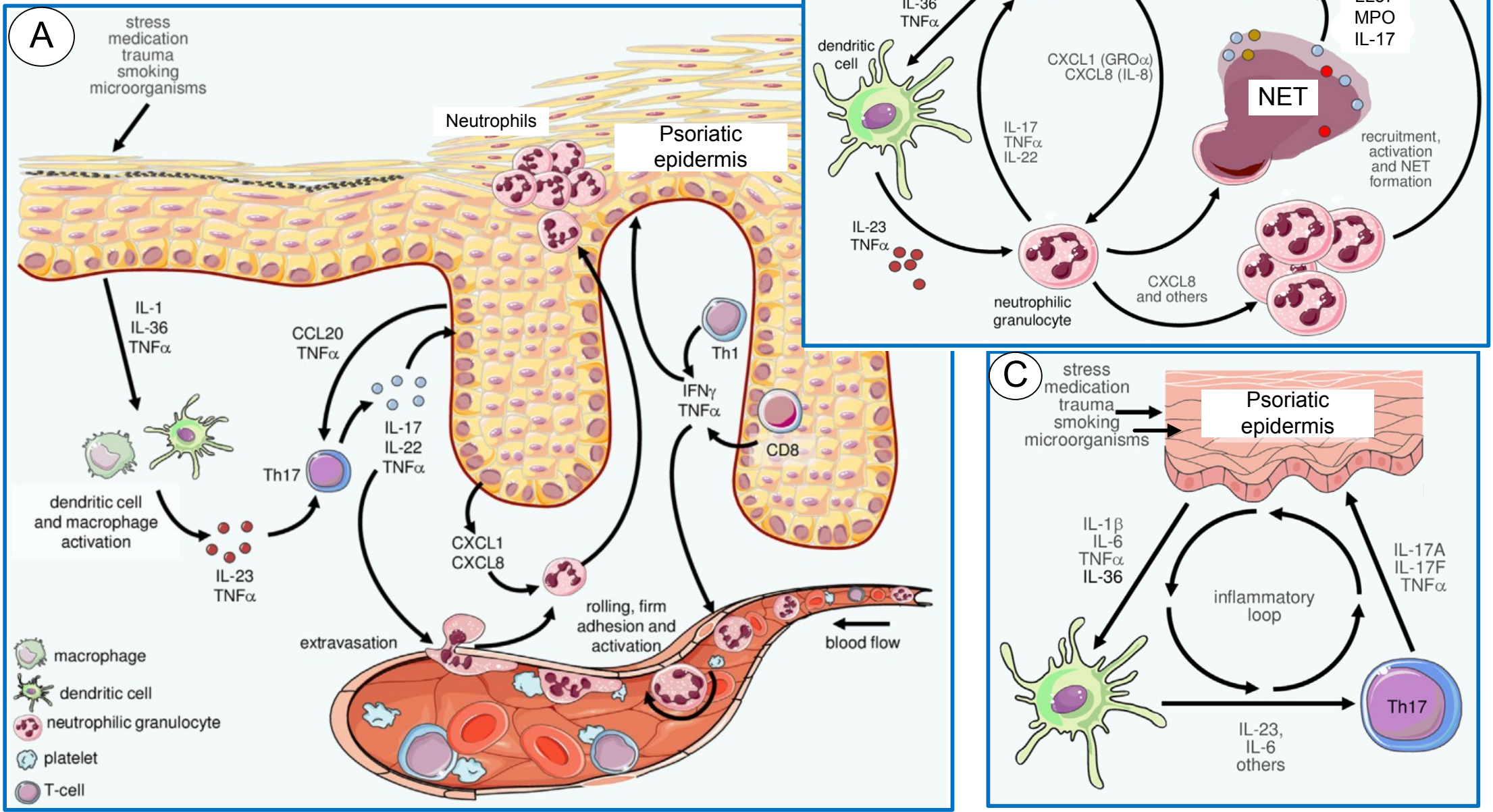
Fig.3 from Delgado-Rizo V, Martínez-Guzmán MA, Iñiguez-Gutierrez L. et al. Neutrophil Extracellular Traps and Its Implications in Inflammation. Front Immunol. 2017 Feb 6;8:81. 28220120.

Model of psoriasis pathogenesis taking into account NET (Schon 2018)

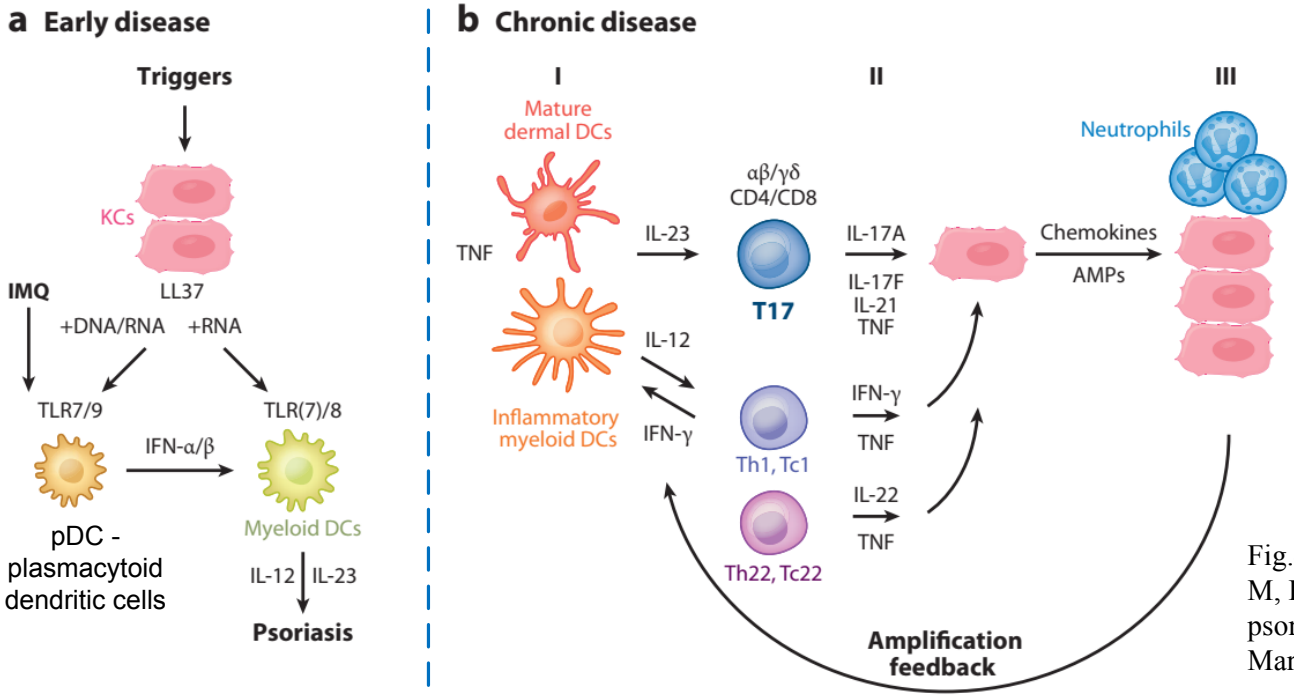
The work was carried out by Schön MP, Erpenbeck L., University Medical Center Göttingen and University of Osnabrück, Germany.

Fragments of Fig.3 and Fig.4 from Schön MP, Erpenbeck L. The interleukin-23/ interleukin-17 Axis Links Adaptive and innate immunity in Psoriasis. Front Immunol. 2018 Jun 15;9:1323. 29963046.

B and C - specification of processes featured in A.



Model of psoriasis pathogenesis without NET (Lowes 2014, Hawkes 2017)



Both studies were carried out in Laboratory for Investigative Dermatology, Rockefeller University, New York, NY, USA, supervised by James G. Krueger. At the same place, GK-model of pathogenesis was developed.

Fig.4 from Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol. 2014 Mar 21;32:227-55. 24655295.

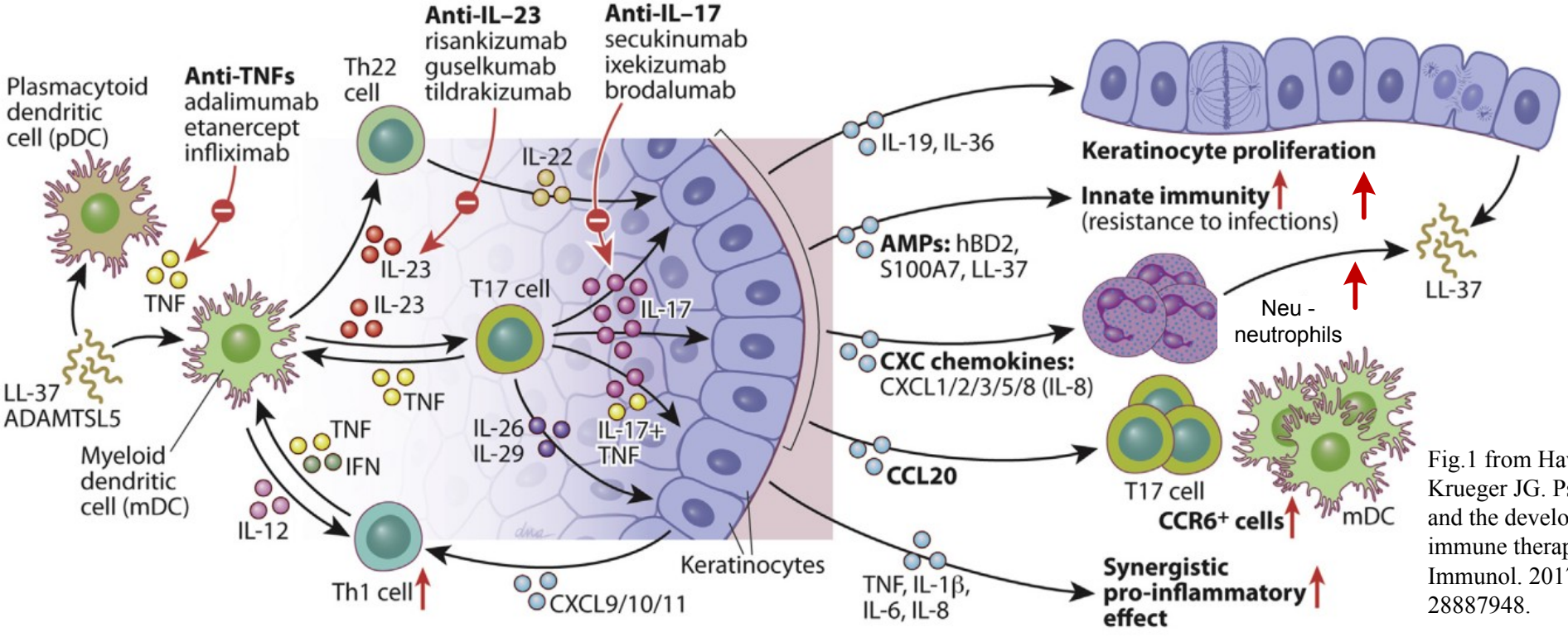


Fig.1 from Hawkes JE, Chan TC, Krueger JG. Psoriasis pathogenesis and the development of novel targeted immune therapies. J Allergy Clin Immunol. 2017 Sep;140(3):645-653. 28887948.

Model of psoriasis pathogenesis without Neu and NET (Benhadou 2018)

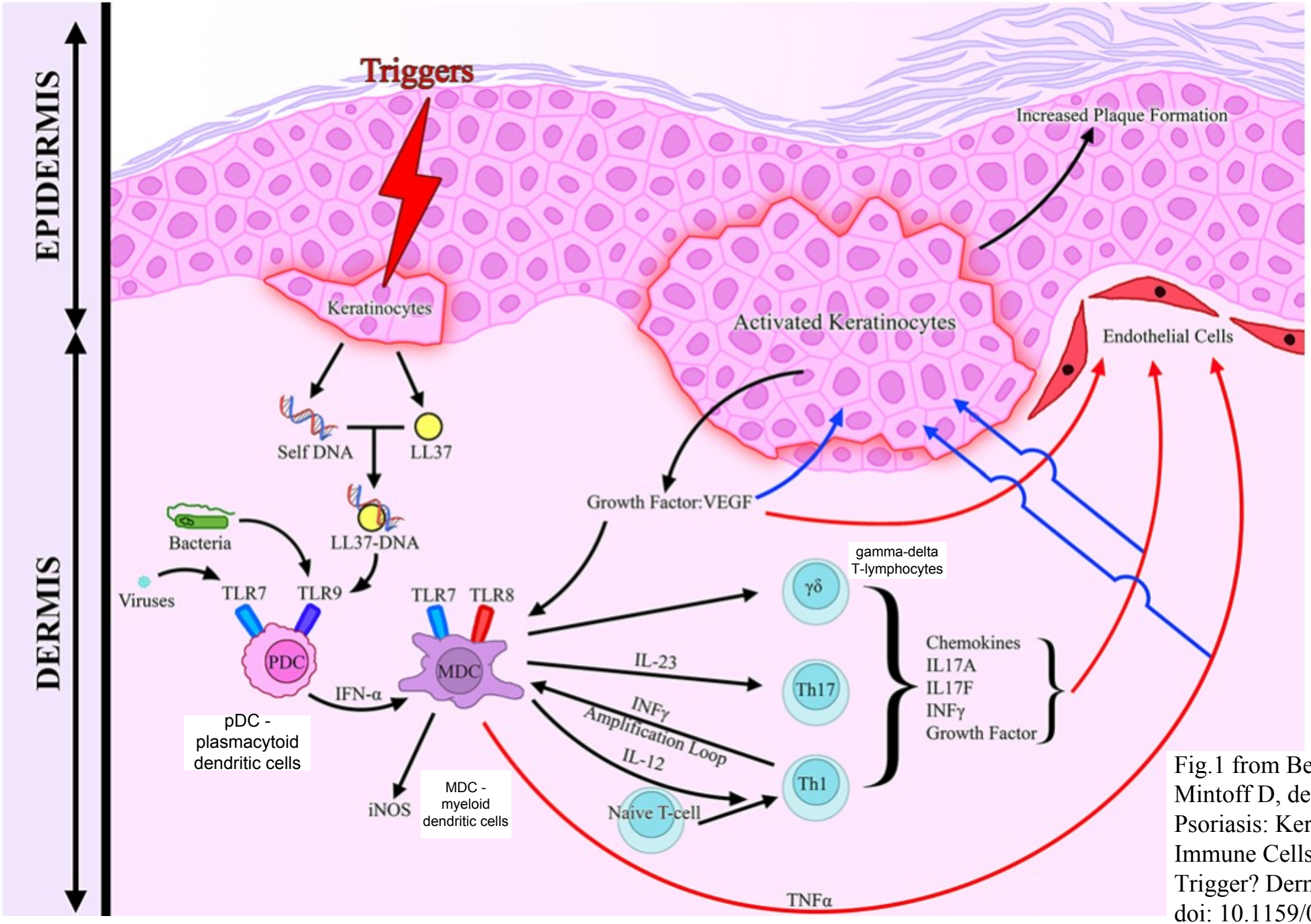


Fig.1 from Benhadou F, Mintoff D, del Marmol V Psoriasis: Keratinocytes or Immune Cells – Which Is the Trigger? Dermatology 2018. doi: 10.1159/000495291 .

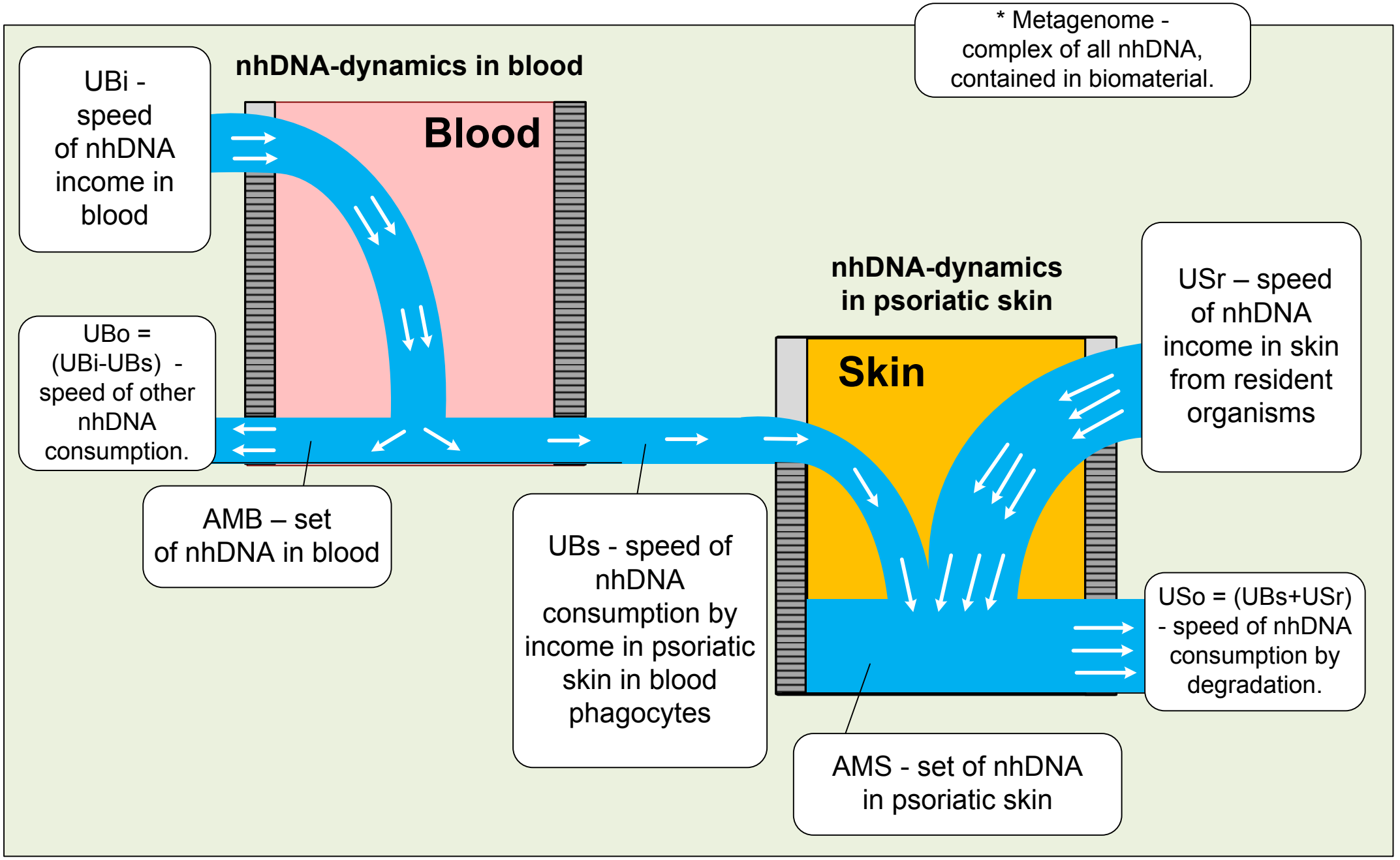
Metagenomes of blood and psoriatic skin. Research project.

Section 4.

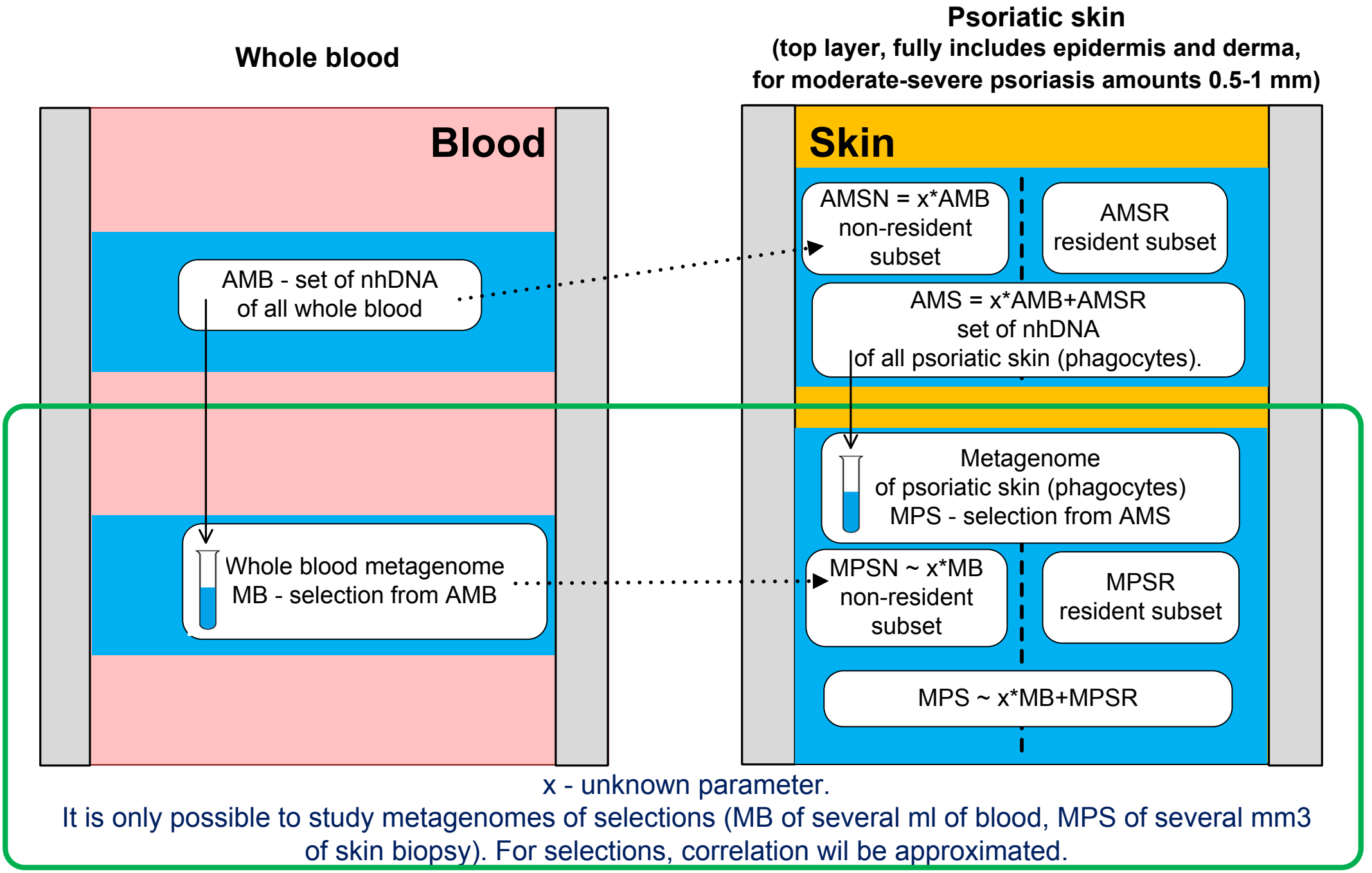
Complex study of metagenomes of blood and psoriatic skin.

Methods and problems of host DNA elimination.

Whole blood metagenome* and metagenome of psoriatic skin (phagocytes) in dynamics



Whole blood metagenome and metagenome of psoriatic skin (phagocytes) at its stable state. Instant cut.



x - unknown parameter.

It is only possible to study metagenomes of selections (MB of several ml of blood, MPS of several mm3 of skin biopsy). For selections, correlation wil be approximated.

Presumed fractions of MPS – metagenome of psoriatic skin (phagocytes)

Mixed fraction RuN. Possible causes of existence:

- blood biomaterial contamination by skin microbiome during venipuncture.
- transport of microbiome and/or its nhDNA from skin into blood during trauma and/or infectious inflammation of derma
- presence of identical strains in skin microbiome and GIT (URT) microbiome
- mapping of different species on one reference species

General fraction M. It is assumed that considerable proportion of MB will be found in it (100%?).

MB – Whole blood metagenome

R. Resident.
nhDNA of resident origin - only from skin microbiome. (present in MPS, but not present in MB).

RuN. Mixed.
nhDNA of resident and non-resident origin.
(Everything from fraction M which not included in fraction N. For each nhDNA of this fraction, subsets of resident and non-resident origin are determined algorithmically).

M. General. (nhDNA present both in MB and in MPS).

N. Non-resident. nhDNA of non-resident origin.
(This nhDNA appeared in MPS only because it got into psoriatic skin in blood phagocytes. Is determined logically and algorithmically. Originally, this fraction logically includes all definitely non-resident nhDNA).

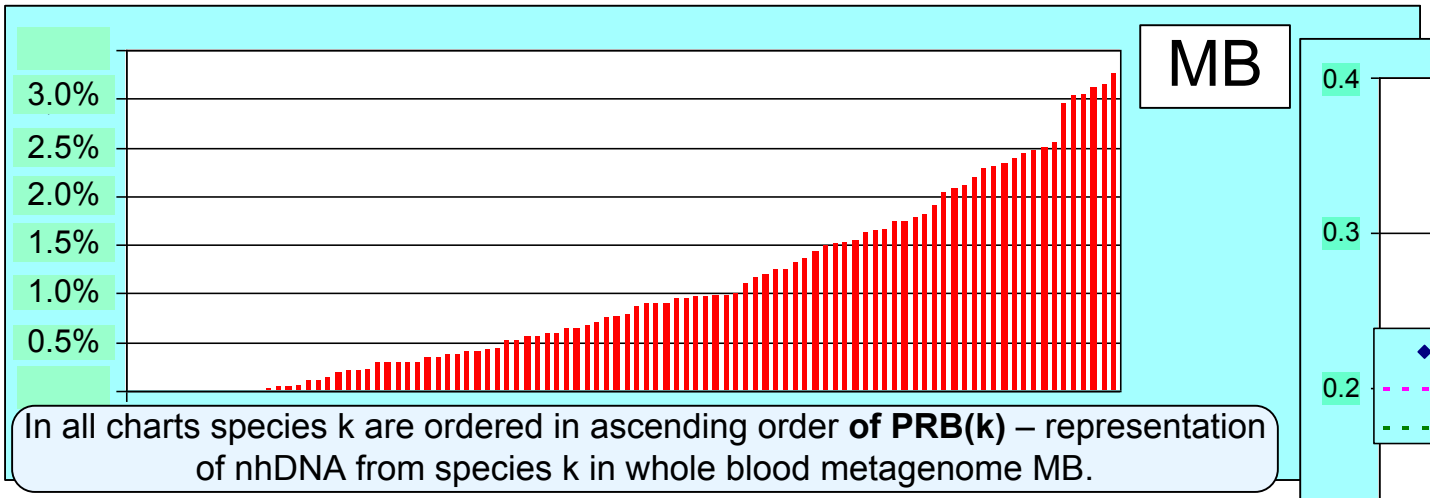
nhDNA present in MB, but not present in MPS
(possible for nhDNA negligibly represented in MB).

MPS - Metagenome of psoriatic skin (phagocytes)

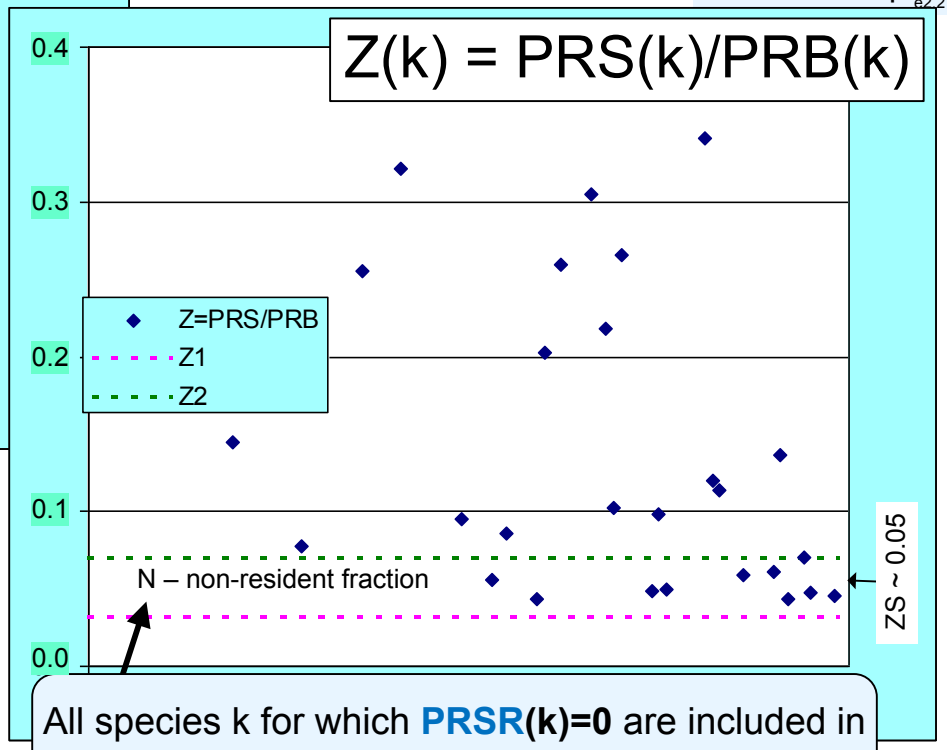
nhDNA - non-host DNA (including bacDNA)

* those nhDNA whose representation is more than 0.01% are considered (the value is conventional).

MPS division algorithm - metagenome of psoriatic skin (phagocytes) into fractions and subsets. Example.

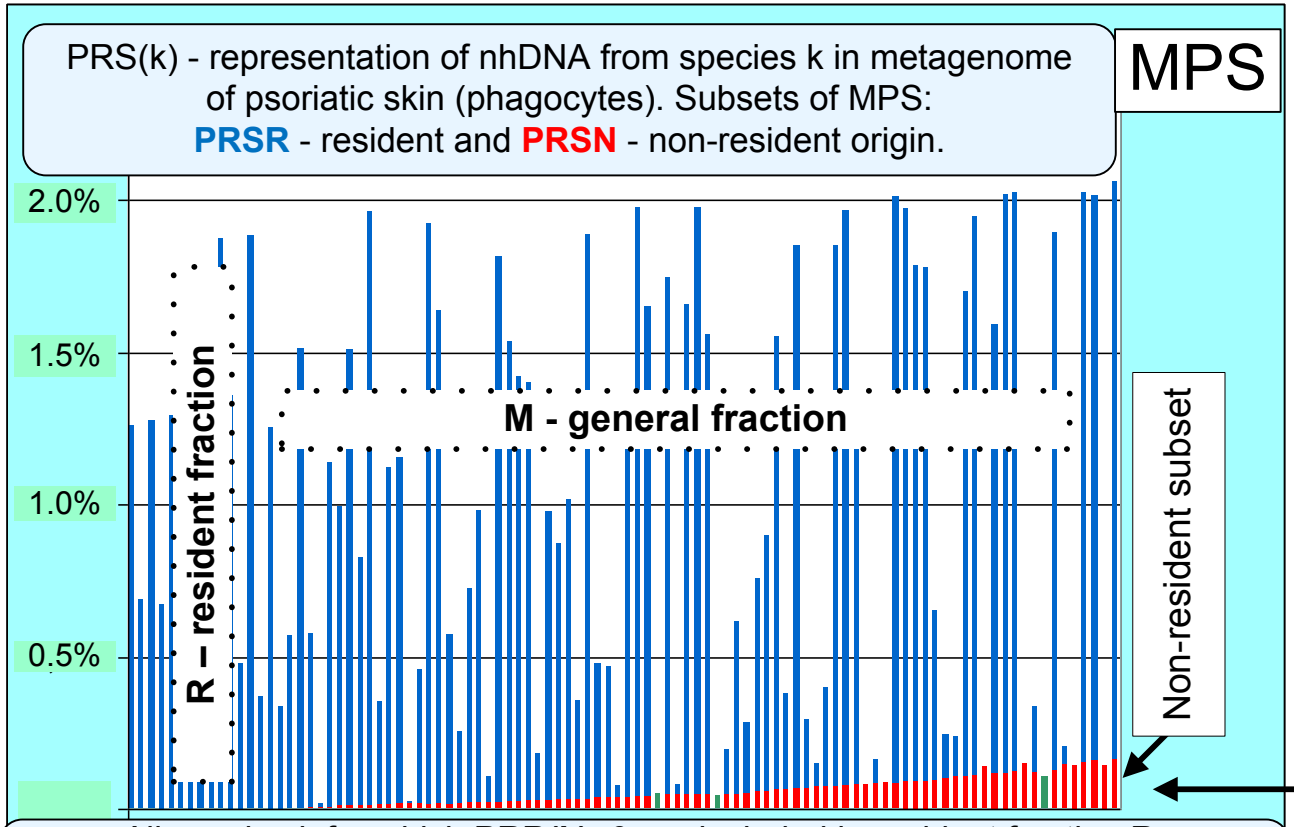


In all charts species k are ordered in ascending order **of PRB(k)** – representation of nhDNA from species k in whole blood metagenome MB.



All species k for which **PRSR(k)=0** are included in non-resident fraction N.
For these k value Z(k) = PRS(k)/PRB(k) - between two dashed lines.
For species k included in the resident fraction, values Z(k) are not calculated since **PRB(k)=0**.
Values Z(k) over 0.4 are not given.

PRS(k) - representation of nhDNA from species k in metagenome of psoriatic skin (phagocytes). Subsets of MPS:
PRSR - resident and **PRSN** - non-resident origin.

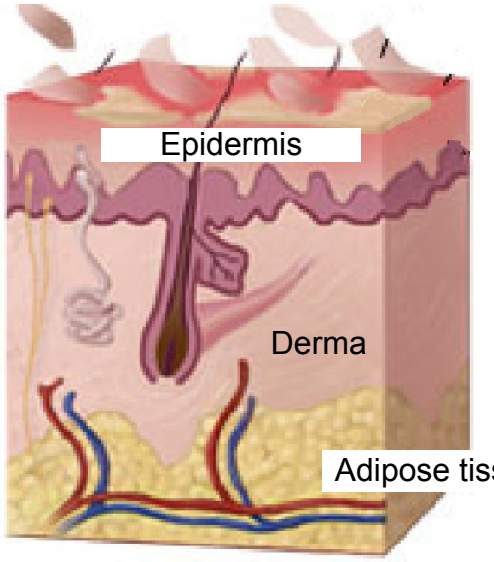


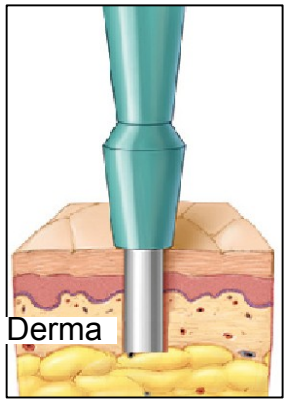
All species k for which **PRB(k)=0** are included in resident fraction R.
These are only blue columns without the red bottom – they are on the left of the chart.

All species k for which **PRSR(k)=0** are included in non-resident fraction N.
These are only the red column (or green - NL) without the blue top, and they are depicted in the chart among the species from mixed fraction RuN (two-color columns).

Smaple on «EX3(G1+,G2+)»

Selection of phagocytes from psoriatic skin by immunomagnetic method





Biopsy homogenization for full division of cells.

Addition of specific antibodies to cellular suspension

Incubation and centrifugation

Addition of magnetic microbeads, incubation

Negative selection

Selection of cells not connected with microbeads

Addition of reagent extricating from microbeads, incubation

Selection of cells extricated from microbeads

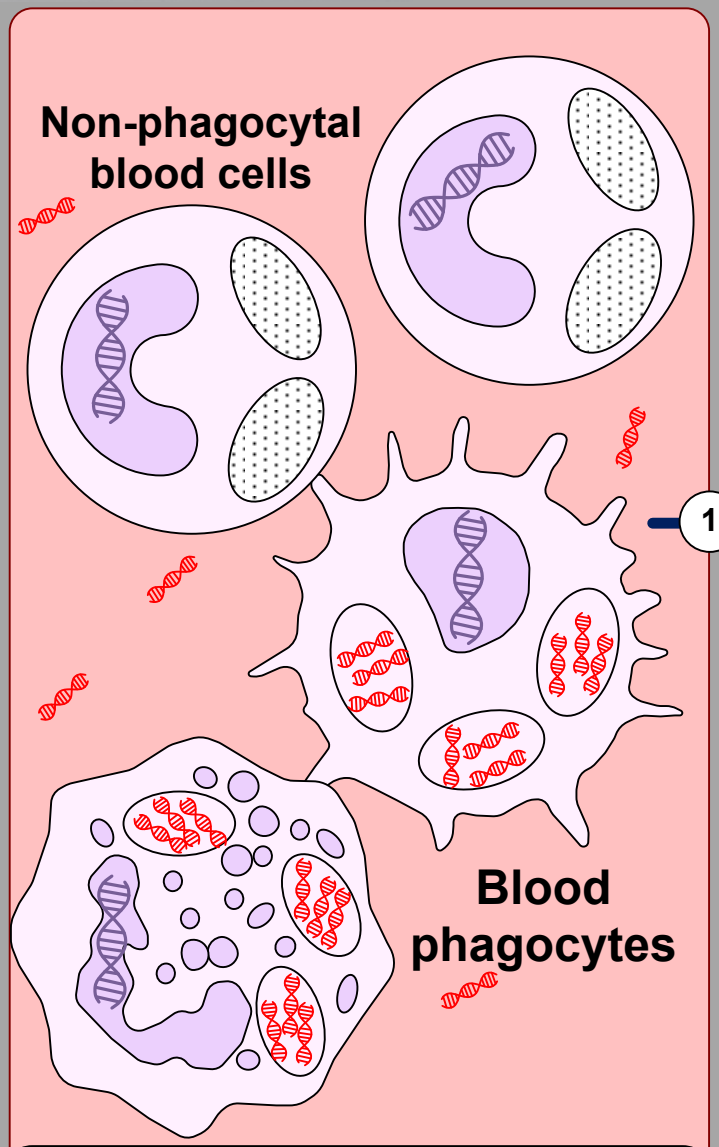
Positive selection

Obtaining biopsy from psoriatic plaque in sterile conditions by means of punch (diameter < =3 mm) on depth (0.5-1 mm), sufficient to fully take epidermis and derma.

Preparation and subsequent enrichment of blood samples by NebNext Microbiome Enrichment kit

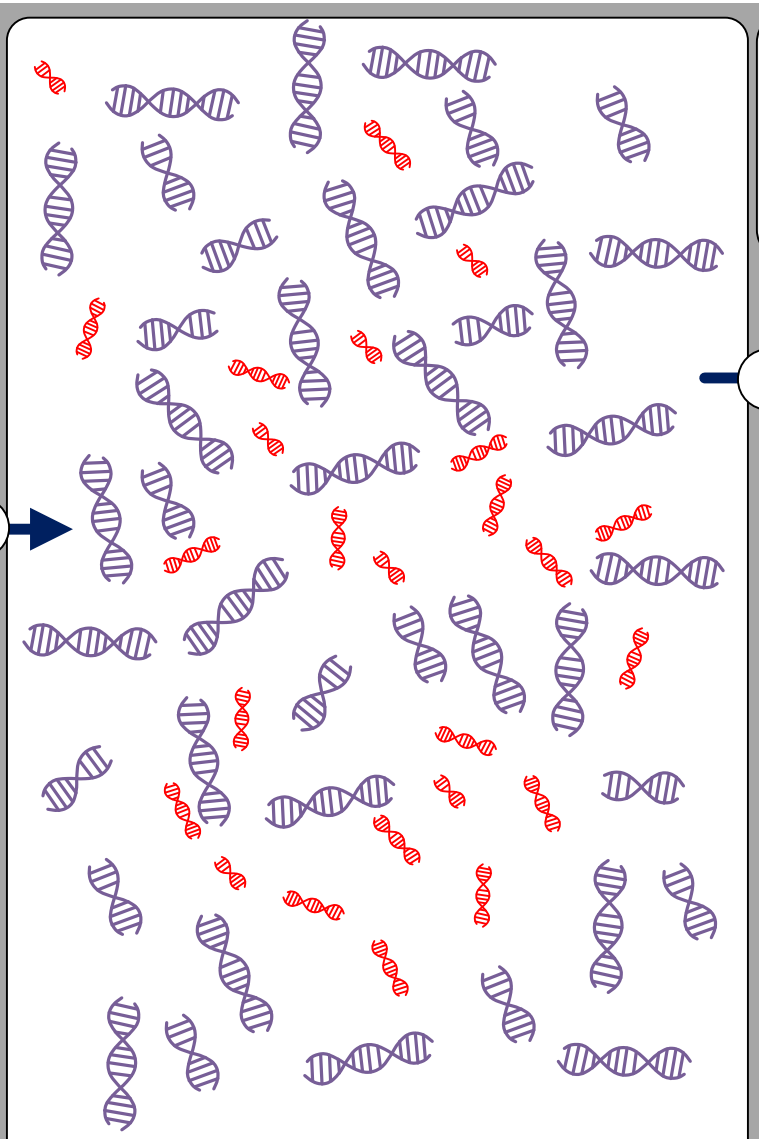
78

NME-1 e2.2



nhDNA and other non-host biomaterial continually endocyted by blood phagocytes. Inside phagocytes these is 2-3 orders more nhDNA than in plasma.

1

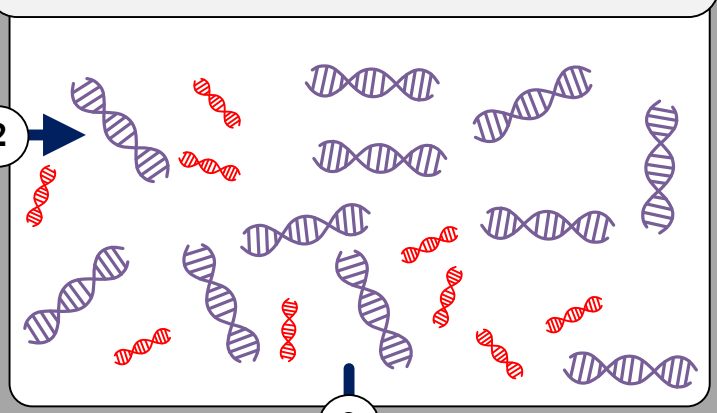


1

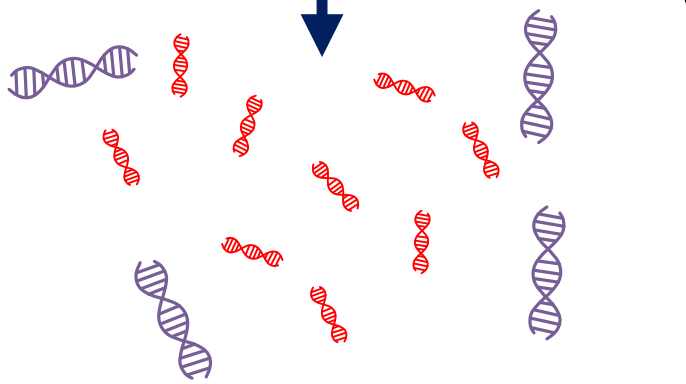
Isolation of all DNA from whole blood. hDNA constitutes more than 99% (in weight).

2

Excluding DNA fragments with the size <= 15 KB (electrophoresis on agarose gel). nhDNA composition are practically does not change.



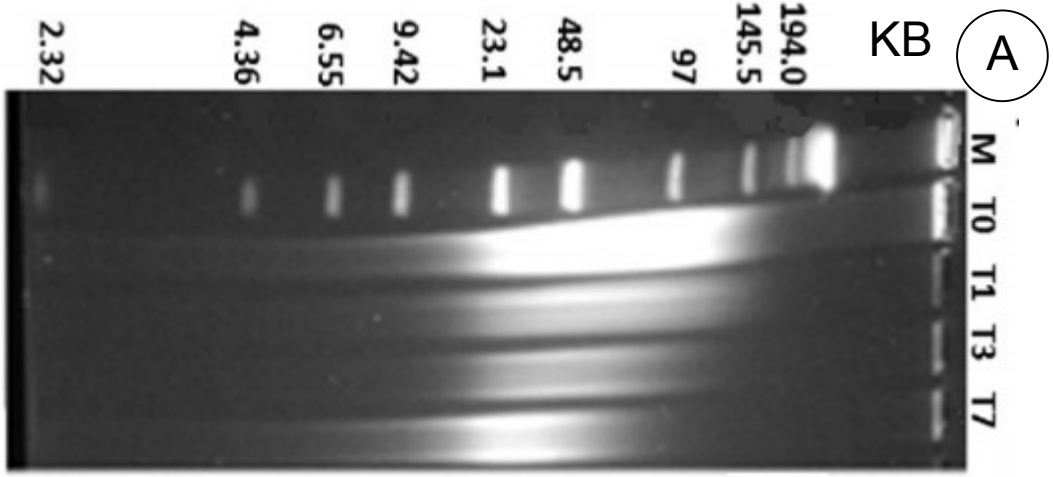
3



3

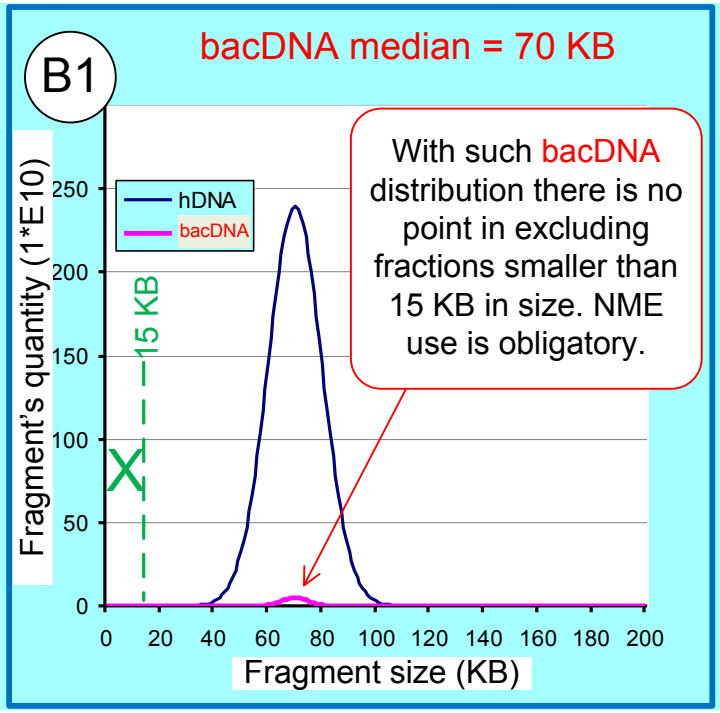
Selective elimination of hDNA fragments. That done, hDNA constitutes no more than 5-35% (in weight). Content and composition of nhDNA practically do not change.

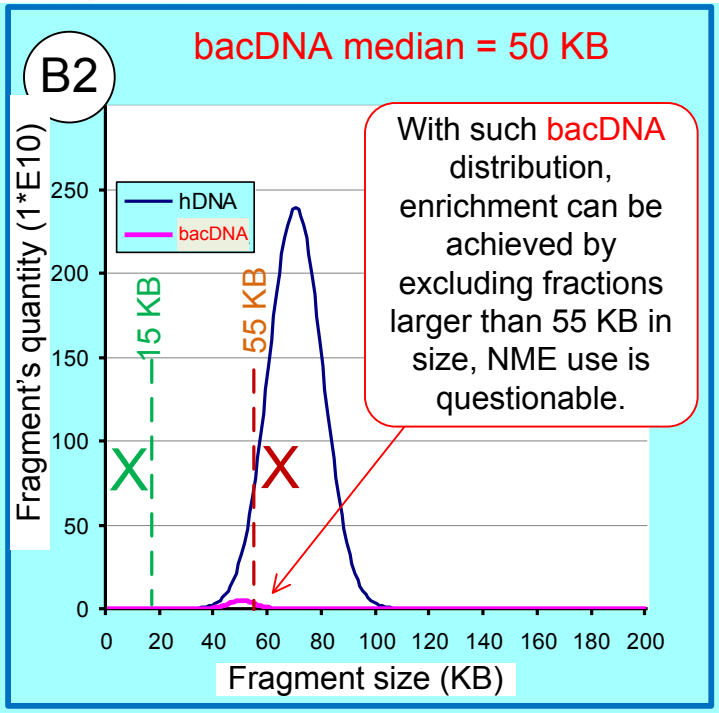
Integrity (fragment distribution) of DNA isolated from whole blood

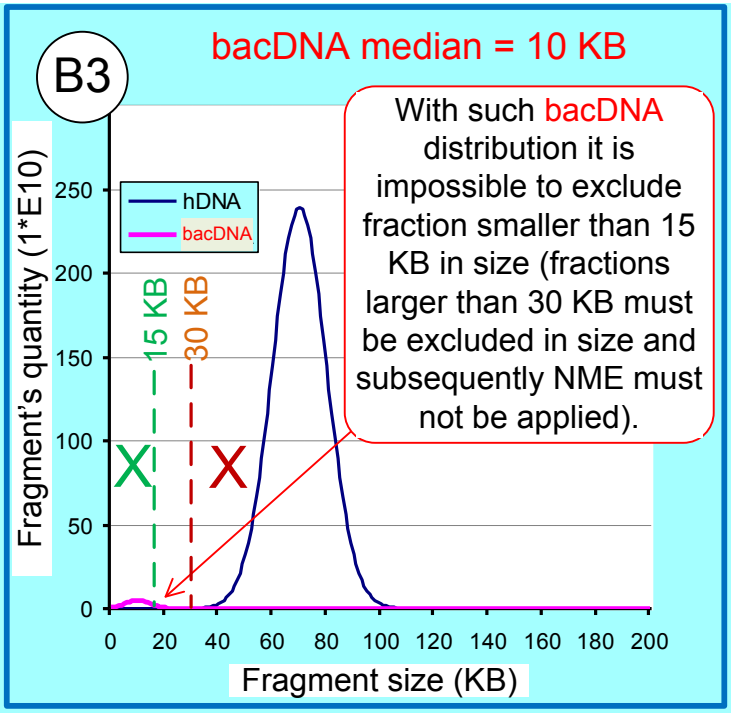


Integrity of DNA (PFGE). Isolation by Gentra Pure Gene Blood kit (Qiagen).
M = marker; T0 =immediately after sampling; T1 = in 1 day; T3 = in 3 days; T7 = in 7 days.
According to kit description, fragments must mainly be from 100 to 200 KB. There must not be fragments smaller than 50 KB in size.

Fig.1A from Malentacchi F, Ciniselli CM, Pazzagli M. et al. Influence of pre-analytical procedures on genomic DNA integrity in blood samples: the SPIDIA experience. Clin Chim Acta. 2015 Feb 2;440:205-10. 25485853.



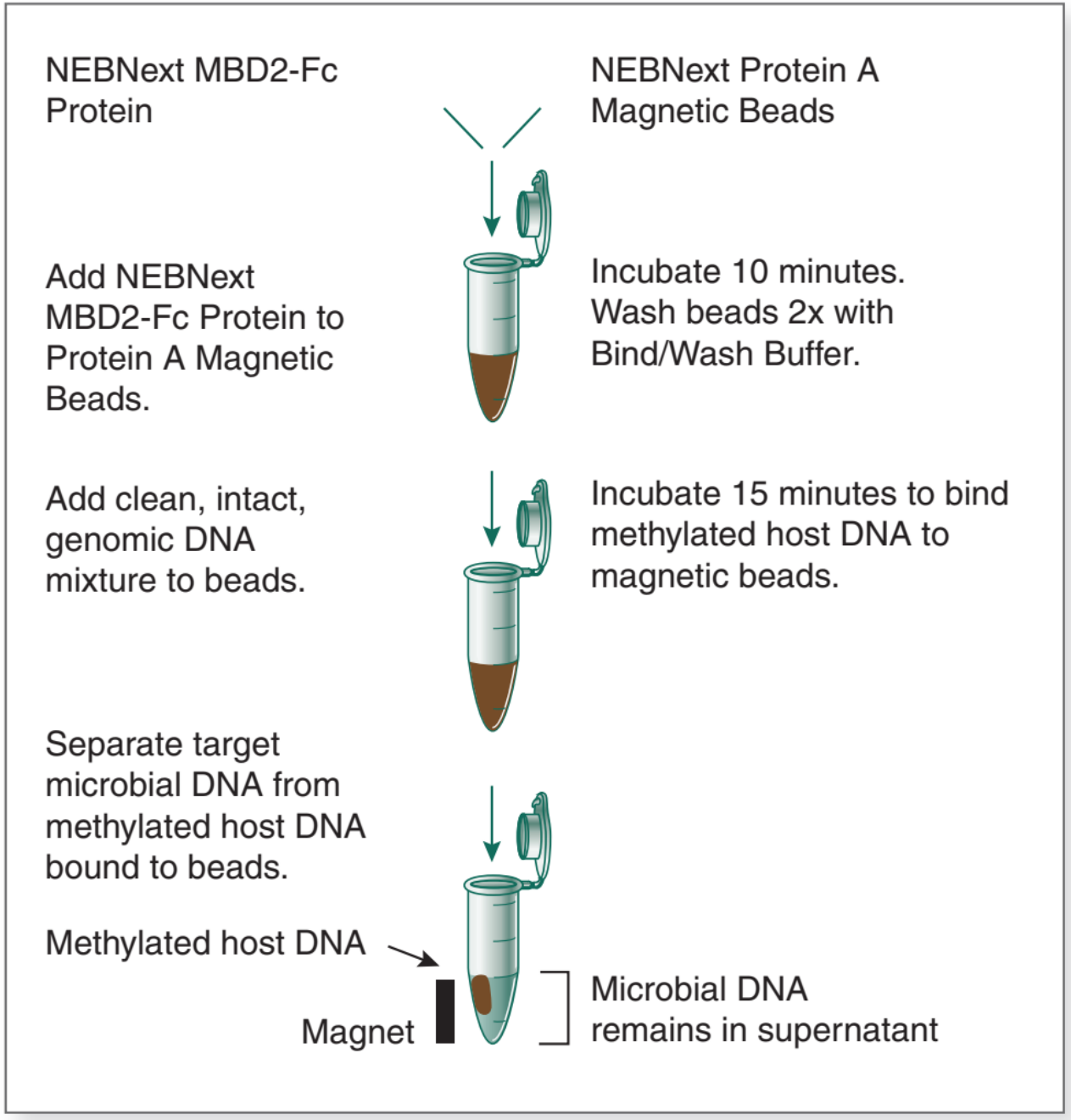




Integrity (fragment distribution) of all DNA isolated from 1 ml of whole blood (according to T0 in the photo - median ~70 KB, deviation ~10 KB);
For bacDNA - distribution is unknown, the amount of bacDNA in charts for descriptive reasons constitutes 1% of all DNA. According to known results, it constitutes from 0.03% to 0.2%).
Excluding fragments smaller than 15 KB - a requirement for subsequent NME use.

Non-host DNA selection from biomaterial with predominant host DNA content (blood or skin cells).

NebNext Microbiome Enrichment Kit



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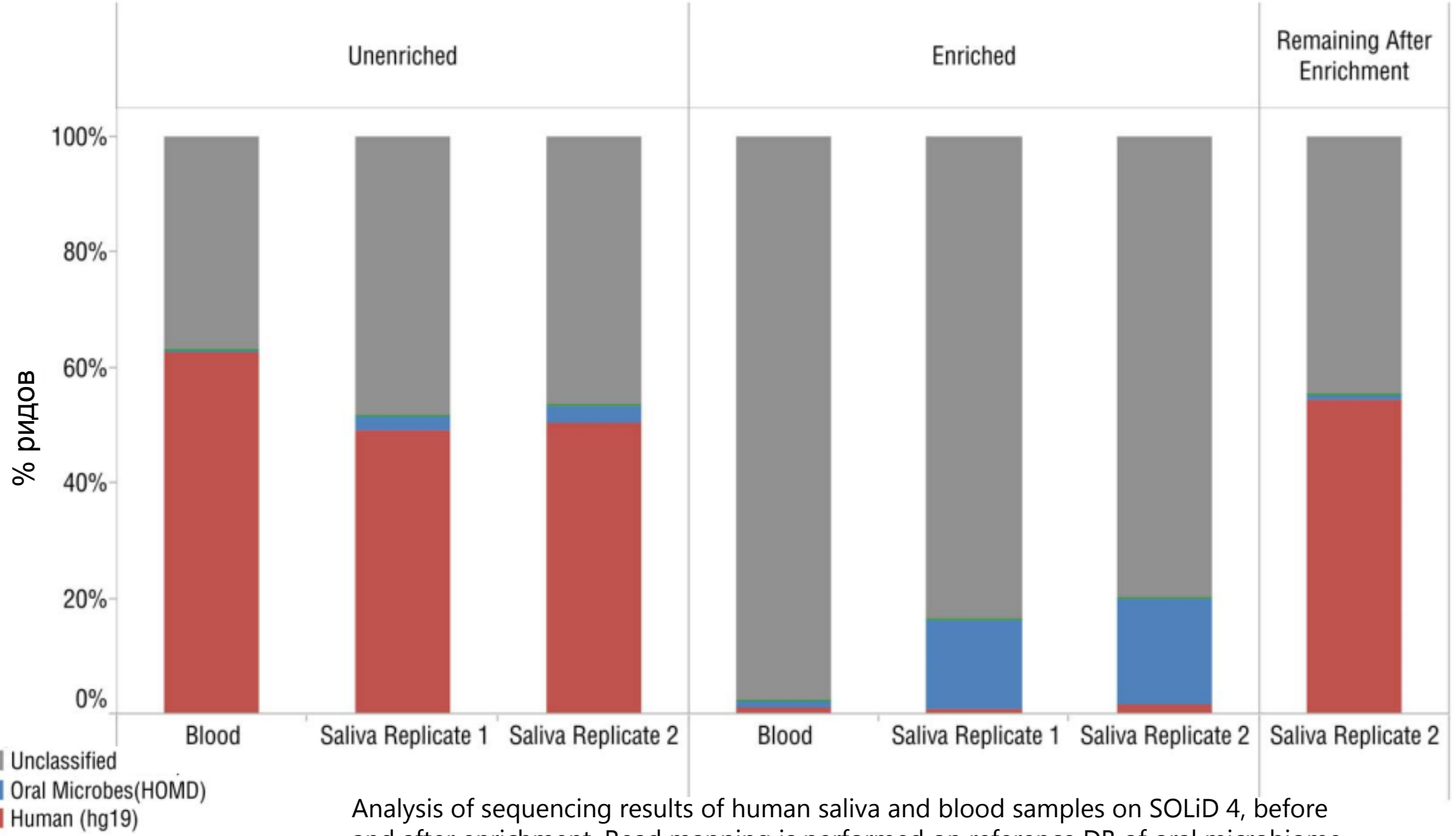
Enrichment of saliva and blood samples by NebNext Microbiome Enrichment kit

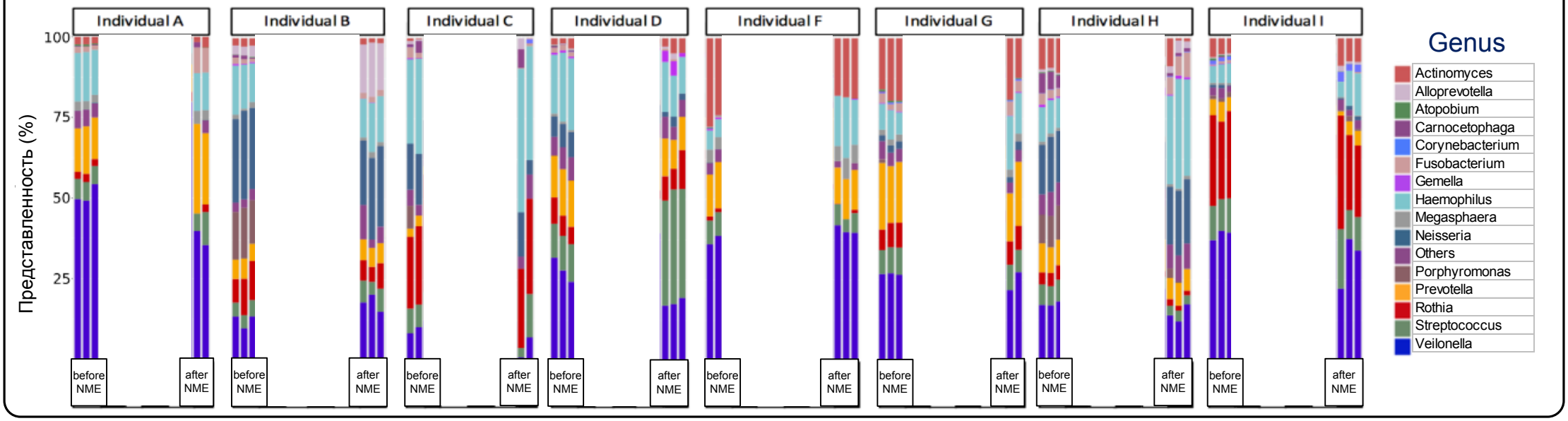


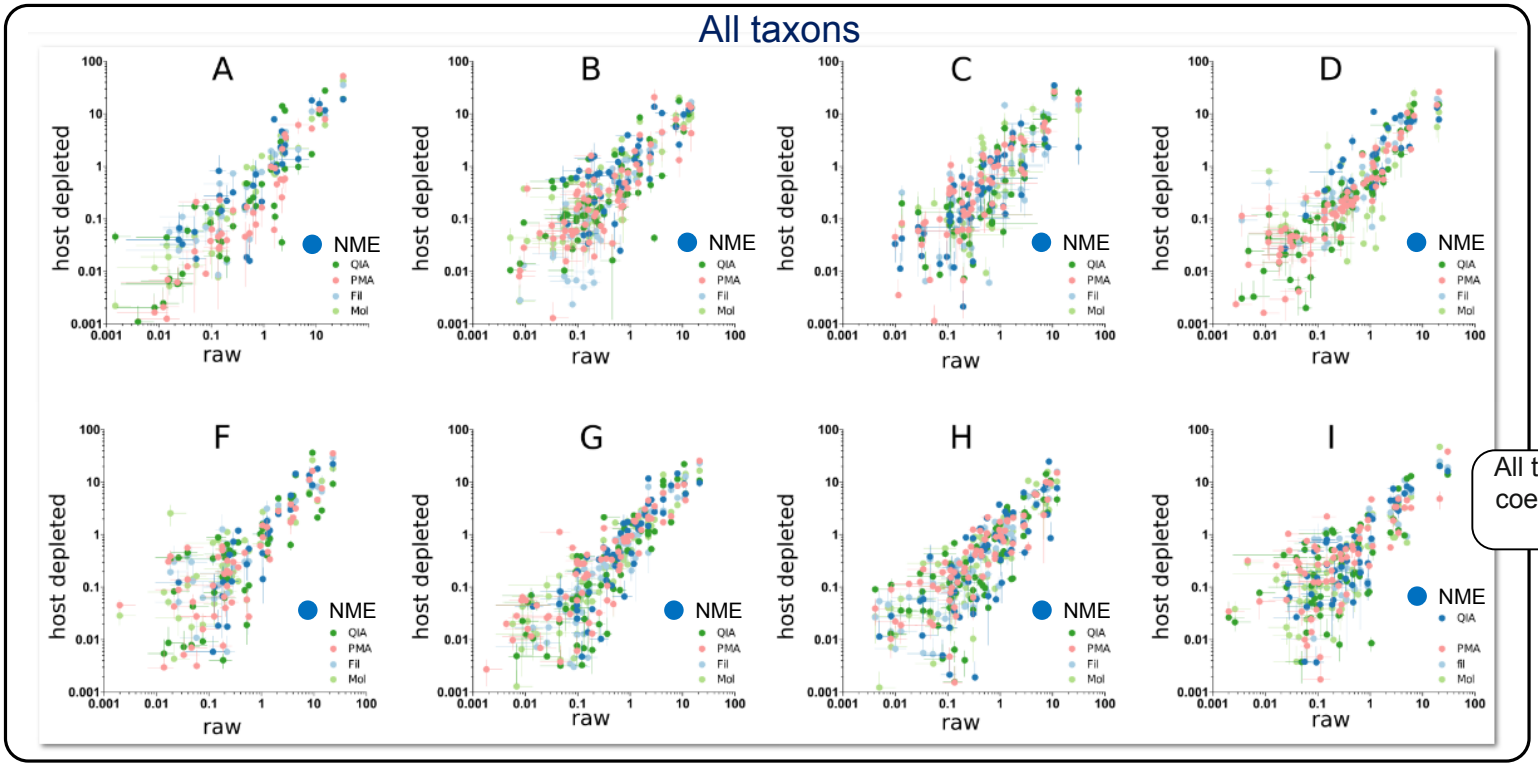
Analysis of sequencing results of human saliva and blood samples on SOLiD 4, before and after enrichment. Read mapping is performed on reference DB of oral microbiome HOMD.

Fragment of Fig.5 from Feehery GR, Yigit E, Oyola SO. et al. A method for selectively enriching microbial DNA from contaminating vertebrate host DNA. PLoS One. 2013 Oct 28;8(10):e76096. 24204593.

NEW ENGLAND BioLabs® Inc.

hDNA elimination from saliva samples in several ways, including NME (HP, n=8, Marotz 2018)





Saliva samples were taken from 8 HP, WMS-test was performed three times for each sample: before and after hDNA elimination.

(in the top chart the information on other ways of hDNA elimination are covered for highlight NME)

All taxons. Stirmen correlation coefficient for NME amounted to 0.75 ± 0.13.

Based on Fig. S4 and Fig. S5 from Marotz CA, Sanders JG, Zuniga C. et al. Improving saliva shotgun metagenomics by chemical host DNA depletion. Microbiome. 2018 Feb 27;6(1):42. 29482639.

Metagenomes of blood and psoriatic skin. Research project.

Section 5.

Order of patients' participation.

Main questions and novelty.

Order of participation of psoriatic patients (PP) and healthy persons (HP) in NCS1 project.

Stage 1-1. Selection and preparation.

Informing, questioning, collecting data on PPC (PP - candidates for participation) and HPC (HP - candidates for participation). Selection of PPC having minimum health problems (apart from psoriatic disease). Selection of HPC without any heatth problems. Among those allowed to participate there must be PP with a wide range of PASI (from weak to heavy). The decision on primary selection is made by a expert council. IEMC (integrated electronic medicine card) is formed for each participant. Consultation by dermatologist. Control blood tests. The final decision on including PPC and HPC in the project is made by a expert council.

Stage 1-2. Protocol development, ordering materials and kits.

Stage 1-3. Pilot research for PP blood samples.

Optimization of patients preparation and protocol optimization to maximize bacDNA concentration.
Assessment of bacDNA-test for small intestine permeability. Minimization of bacDNA concentration in NTC (no template controls).

Stage 1-4. Re-examination and selection of PP and HP. Biomaterial sampling.

Consultation by a dermatologist (for determine up-to-date health of PP and HP and to specify dates for biomaterial sampling). Specifying a single date for biomaterial sampling. Sampling and preprocessing of biomaterials.
Blood sample from PP and HP, skin sample from PP only.

Stage 1-5. Identifying and studying whole blood metagenome and PAMP-nemia.

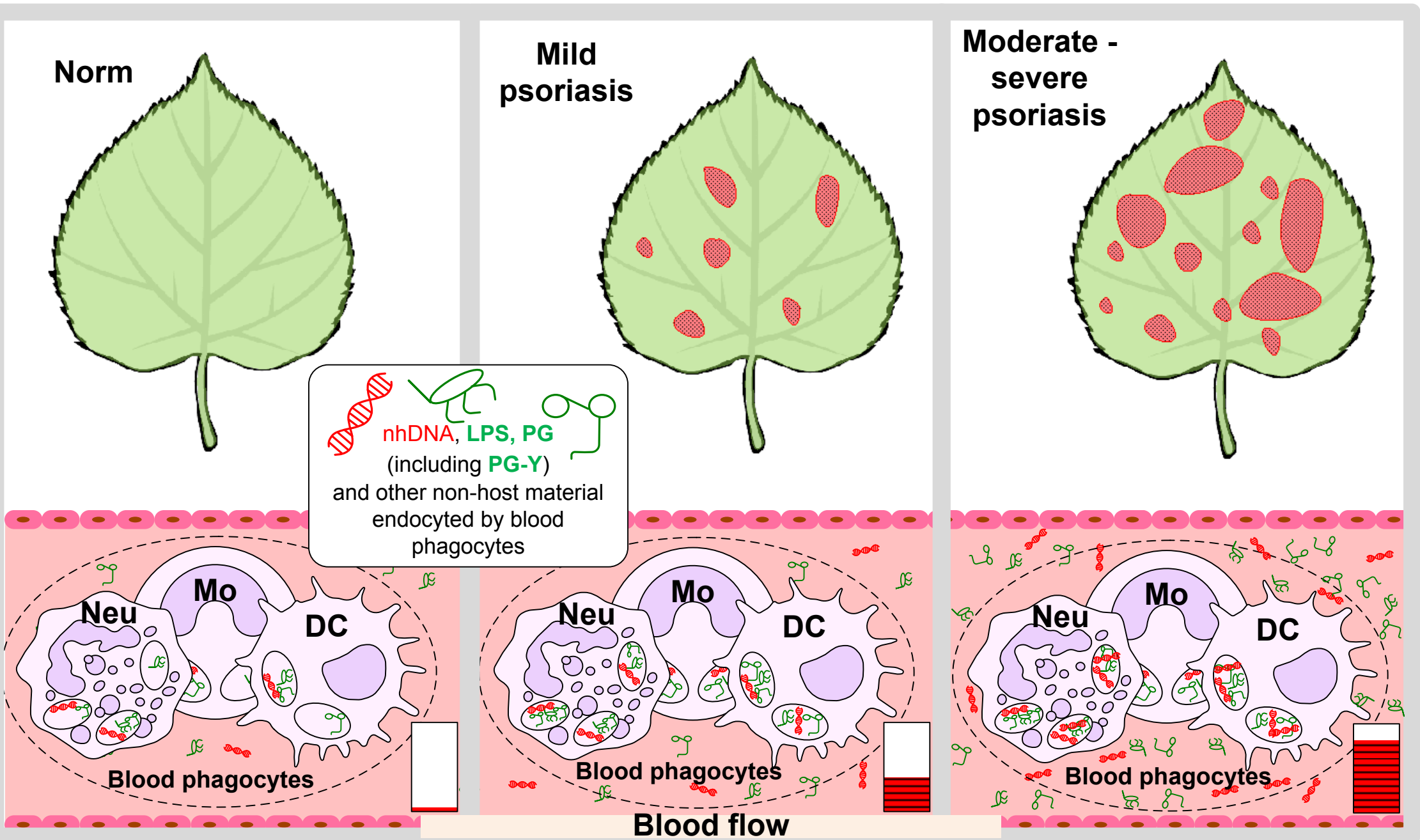
Identifying whole blood metagenome (WMS-test) and determining nhDNA concentration.
Determining PAMP-nemia. Determining macromolecular small intestine permeability. Search of correlations between PASI and characteristics of whole blood metagenome and PAMP-nemia. Statistical analysis and assessment of results.

Stage 1-6. Identifying metagenome of psoriatic skin (phagocytes). Complex study of metagenomes.

Identifying and studying metagenome of psoriatic skin (phagocytes) (WMS test).
Complex study of metagenomes of whole blood and psoriatic skin (phagocytes),
search of interrelations. Statistical analysis and assessment of results. Summing up Stage 1.

Stage 1-5

Question 1. Does severity of psoriatic disease correlate with concentration of any nhDNA in whole blood and/or with PAMP-nemia level?



Question 2. Does non-degraded nhDNA come from blood into psoriatic skin?

Skin

Norm

MF
MoDC
RSP
MoDP

RSP - Resident Skin Phagocytes
(smaller size - convention)

All skin phagocytes endocytose nhDNA, **LPS, PG** (including **PG-Y**) and other non-host biomaterial of resident origin (i.e. from any microorganisms living on skin and in skin).

Mild psoriasis

Do nhDNA, **LPS, PG** (including **PG-Y**) and other non-host biomaterial come into psoriatic skin from blood flow in blood phagocytes?

Moderate - severe psoriasis

Neu
Mo
DC
2

Non-resident skin phagocytes.
(bigger size - convention).

Blood phagocytes
in norm are not attracted in skin

Blood phagocytes

Blood phagocytes
intensively are attracted to inflammatory skin

Blood flow

Project NCS1. Two main questions.

Stage 1-5

Question 1. Does severity of psoriatic disease correlate with concentration of any nhDNA in whole blood and/or with PAMP-nemia level?

Stage 1-6

Question 2. Does non-degraded nhDNA come from blood into psoriatic skin?
If so, which part of whole blood metagenome is found in metagenome of psoriatic skin (phagocytes) and in what concentration?

Reinvestigation at the research performer's expense under tightened control.

YES

Was contamination level in WMS-tests higher than admissible?

NO

Additional research.
Determining GRS (genetic risk score) for psoriatic patients. Search of correlations between PASI and combined parameters: GRS and concentration of any nhDNA in whole blood.
Are correlations present?

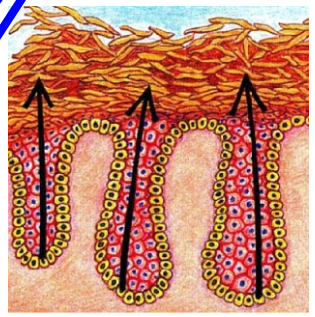
YES

NO

Brainstorming.
Content updating of Stage 1-6 through the lens of the obtained results.

NO

YES



Repeated researches at expense of contractor under more rigid control.

YES

NO

Contamination level in WMS-tests was higher than admissible?

NO

YES

Is concentration of nhDNA of non-resident origin higher than contamination level?

YES

Preparing and implementing Stage2

NO

Brainstorming.
Content updating of Stage 2 through the lens of the obtained results.

Project NCS1. What novelty consists in?

New idea:

New model of pathogenesis of psoriatic disease (PD).

New methods of research

(at PD and for control healthy group)

Researches to be carried out for the first time

- Parameters of fragment distribution of bacDNA found in DNA-samples from whole blood are determined.
- Whole blood metagenome is identified by whole metagenomic sequencing method.
- Whole blood plastome (as part of its metagenome) is identified.
- Metagenome of psoriatic skin (phagocytes) is identified by whole metagenomic sequencing method (including its non-resident fraction).
- nhDNA concentration in whole blood is determined.
- nhDNA concentration of psoriatic skin (phagocytes) is determined (including of non-resident fraction).
- Complex study of whole blood metagenome and metagenome of psoriatic skin (phagocytes) is carried out.
- Macromolecular small intestine permeability is determined by bacDNA-test.
- Main PAMP concentration in blood is determined.