Introduction à la biologie des parasites
Immunité anti-parasitaire
(2ème partie)

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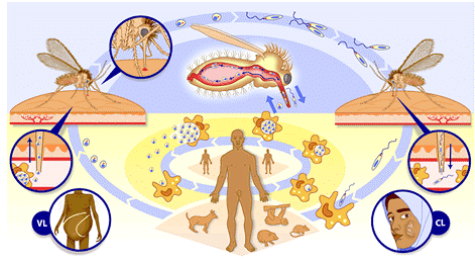
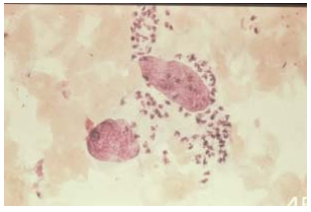
IF2004 IP-d,e,e'
5, 6 et 8 avril 2004

Life cycle of *Leishmania*





Visceral leishmaniasis
- *L. chagasi*
- *L. infantum*

Cutaneous leishmaniasis
- *L. mexicana*
- *L. panamensis*
- *L. amazonensis* (diffuse)
- *L. braziliensis* (muccocutaneous)
- *L. guyanensis*
- *L. peruviana* ...

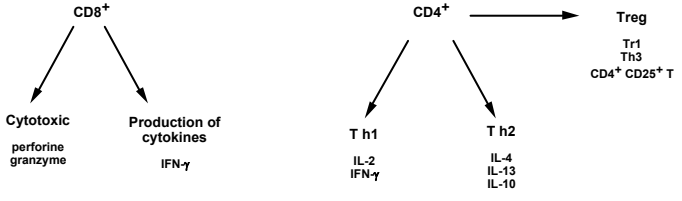
First generation vaccine

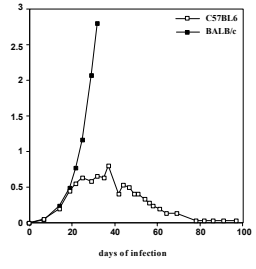
- Vaccine for cutaneous leishmaniasis in the Old World: autoclaved *Leishmania major* (ALM) + BCG
- Vaccine for cutaneous leishmaniasis in the New World: autoclaved *Leishmania amazonensis* (ALA) + BCG
- Vaccine for cutaneous leishmaniasis in the New World: merthiolated *Leishmania amazonensis* (MLA) without BCG
- Vaccine for visceral leishmaniasis in the Old World: ALM + BCG
- Vaccine for cutaneous and visceral leishmaniasis in the Old World: ALM-alum + BCG

Second generation vaccines

glycoprotein 63 kDa (gp63)	r-gp63 dans *Salmonelle*	Partial protection
	r-gp63 dans *BCG*	Partial protection
	peptides +gp63 DNA + adjuvant	Protection
promastigote surface antigen2 (PSA2)	r-PSA2+ *C parvum*	Protection
	PSA2 DNA	Complete protection
Leishmania Activated C Kinase (LACK)	r-LACK + IL-12	Protection
	LACK DNA	Long-term protection
	LACK APL	Protection
Thiol specific oxidant (TSA)	r-TSA + IL-12	Protection
	TSA DNA	Protection
LmSTI1	LmSTI1 DNA	Protection
Cysteine Proteinases (CPI et II)	CPI DNA + CPII DNA	Long-term protection
Histone H1	r-H1	Partial protection

T lymphocytes



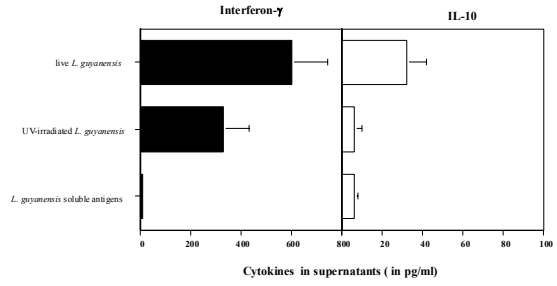


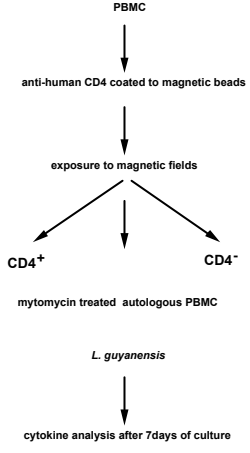
⇒Is Th1/Th2 differentiation occurs in human infected with *L. guyanensis*?

I. Reactivity against *L. guyanensis* in subjects never exposed to *Leishmania*

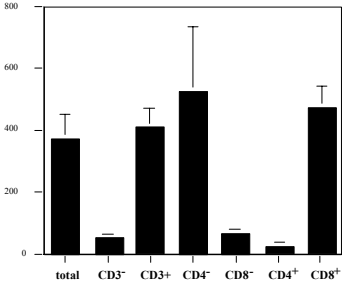
-naïve subjects
- **absence of scars because of leishmaniasis**
- **no history of any stay in country endemic**
- **absence of *L. guyanensis* specific antibodies**
- **no IFN-γ production in response to SLA (<100pg/ml)**

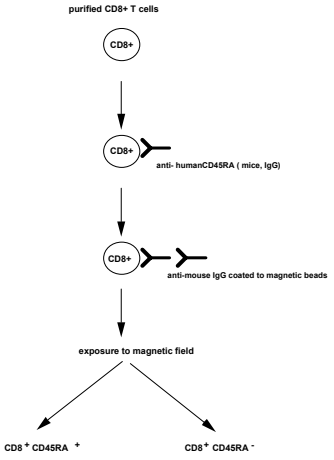
-Lymphocyte culture and cytokine detection



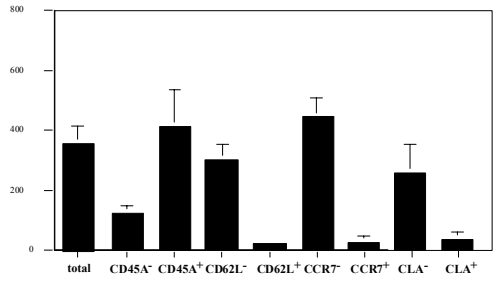


IFN-γ producing cells in response to *L. guyanensis* are $CD8^+$ T cells

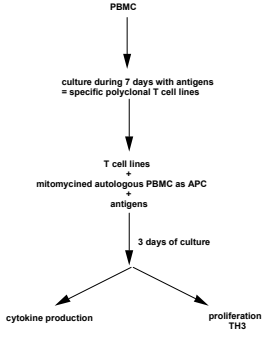




IFN-γ producing CD8$^+$ T cells in response to *L. guyanensis* are "naïve"



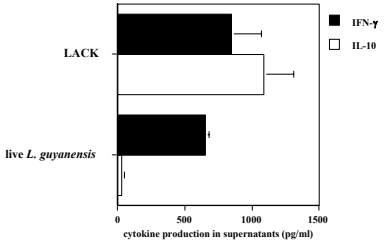
generation of antigen-specific T cell lines and proliferation



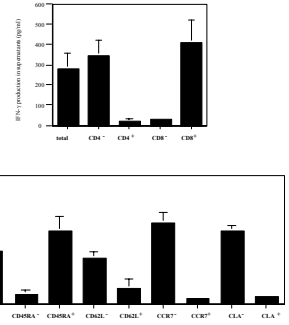
Proliferation and cytokine production of *L.guyanensis* specific T cell lines restimulated with LACK

	Antigens used in restimulation					
	live *L. guyanensis*		LACK		PPD	
T- cell lines (N°)	SI	IFN-γ production (pg/ml)	SI	IFN-γ production (pg/ml)	SI	IFN-γ production (pg/ml)
1	11.7	13590	3.4	7102	1.8	112
2	9.7	39473	7.8	7174	1.9	103
3	9.7	6360	9.4	4741	1.4	225
4	39	13222	4.8	7809	1.3	95
5	20	29205	18	10316	1.6	100
6	18	13574	7.5	7387	0.8	52
7	7	14451	8.5	8458	1.2	36
8	16.8	3791	2.4	1502	0.5	123
9	15.4	9209	15	3658	1.4	114

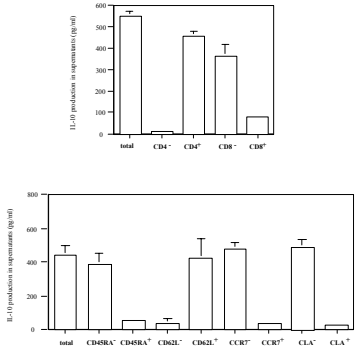
Cytokine production in response to LACK of PBMC from subjects never exposed to *Leishmania*



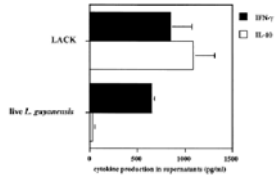
IFN-γ producing T cells in response to LACK are naïve CD8+ T cells



IL-10 producing cells in response to LACK are memory CD4+ T cells



Evidences of the production of suppressive cytokine(s) in response to *L. guyanensis* in PBMC from healthy subjects



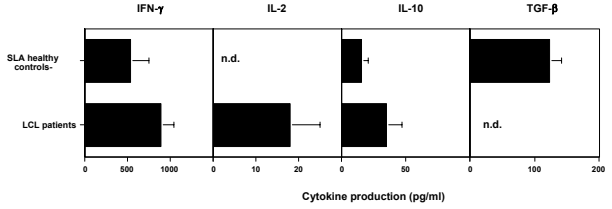
- **IL-10 and IFN-γ producing T cells recognized LACK**
- **Limited IL-10 production in response to live *L. guyanensis***

⇒suppressive cytokines induced by *L. guyanensis* ?

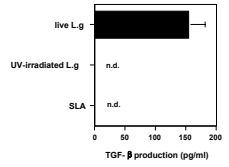
IL-4 and IL-13 not detected

TGF-β?

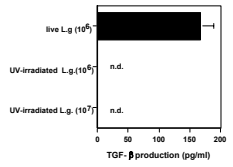
Production of TGF-β1 in response to live *L. guyanensis*



TGF-β1 is produced only in response to live *L. guyanensis*

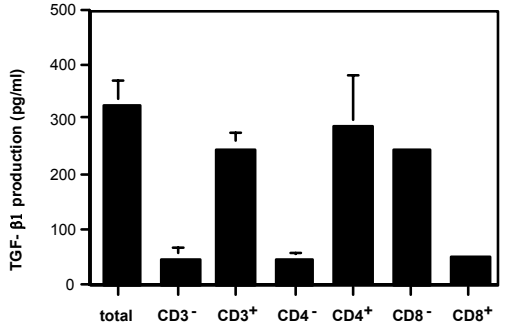


- **TGF-β1 is not produced in response to UV-irradiated *L. guyanensis* (L.g.) and SLA**

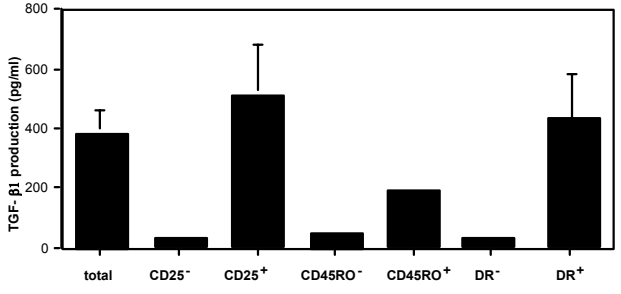


- **TGF-β1 is not produced by stimulation with higher UV-irradiated parasite**

TGF-β1 producing cells in response to live L.g. are CD4⁺ T cells



TGF-β1 producing cells are CD4⁺ CD25⁺ T cells

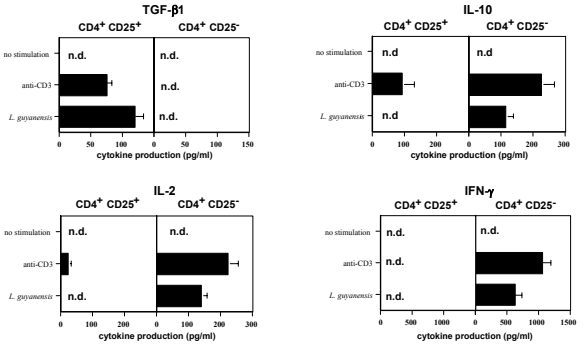


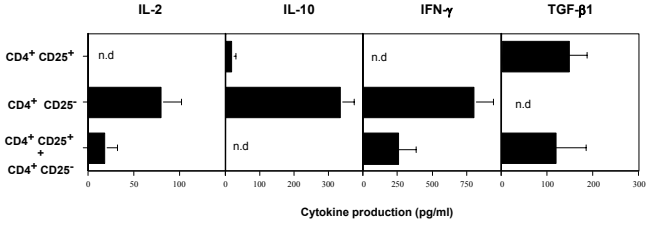
Are the TGF-β1 producing CD4⁺ CD25⁺ T cells T regulatory cells?

Conclusions (1)

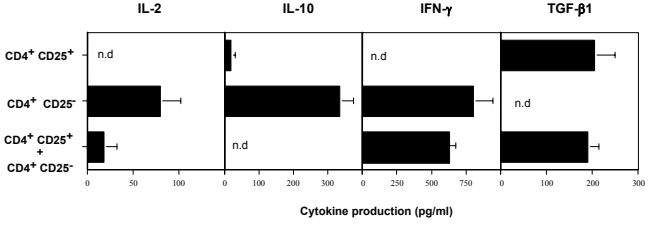
- **Production of TGF-β1 in response to live L.g in healthy subjects**
- **TGF-β1 producing cells are phenotypicaly and functionally identical to the regulatory $CD4^+$ $CD25^+$ T cells**
  - **Expression of CD25, CD45R0, DR surface markers**
  - **Suppressive effects (IL-2 and IL-10 production) on $CD4^+$ $CD25^-$ T cells**
- **are the $CD4^+$ $CD25^+$ activated in the presence of L. g. a sub-population of T regulatory cells?**

$CD4^+$ $CD25^+$ T cells produced TGF-β1 but not IL-10 in response to live L.g.



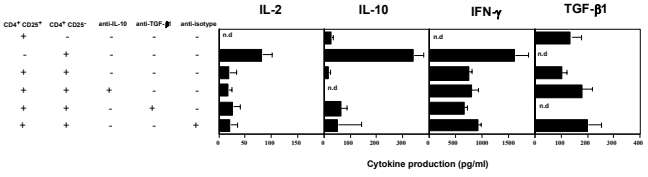


- **$CD4^+$ $CD25^+$ T cells are able to inhibit production of IL-2, IL-10 and IFN-γ by $CD4^+$ $CD25^-$ T cells in the presence of anti-CD3**



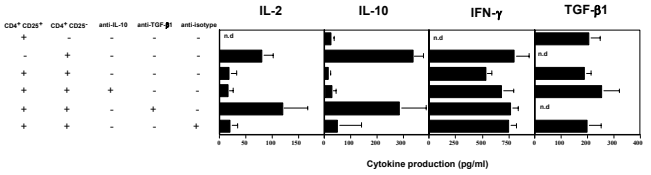
- **$CD4^+$ $CD25^+$ T cells are able to inhibit production of IL-2, IL-10 but not of IFN-γ by $CD4^+$ $CD25^-$ T cells in the presence of live L.g**
  - **⇒ $CD4^+$ $CD25^+$ = Treg cells ?**
  - **⇒ sub-population of regulatory T cells ?**

Role of TGF in the suppressive activity of T regulatory cells on cytokine production in the presence of anti-CD3 ?



addition of anti-TGF-β1 / anti-IL-10 did not reverse suppression of $CD4^+$ $CD25^+$ in the presence anti-CD3

Role of TGF in the suppressive activity of T regulatory cells on the IL-2 and IL-10 production in the presence of live L.g ?



addition of anti-IL-10 did not reverse suppression of $CD4^+$ $CD25^+$ in the presence of live L.g.
addition of anti-TGF-β restore IL-2 and IL-10 production of $CD4^+$ $CD25^-$ T cells in the presence of live L.g

1. Different patterns of cytokines produced in the presence of anti-CD3 and live L.g.

Production of cytokines	In the presence of anti-CD3	In the presence of live L.g.
TGF–β	+	+
IL-10	+	-

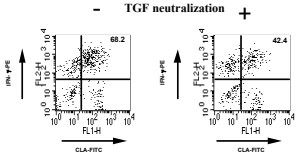
2. Different patterns of suppression of $CD4^+$ $CD25^+$ T cells in the presence of anti-CD3 and L.g

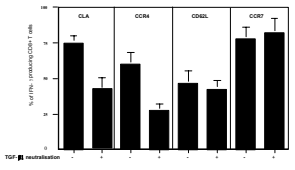
Suppression in the presence of	IL-2	IL-10	IFN-γ
Anti-CD3	+	+	+
Live L.g	+	+	-

3. Different mechanisms of suppression of $CD4^+$ $CD25^+$in the presence of anti-CD3 and live L.g.

Inhibition of suppression in the presence of anti-TGF mAbs	IL-2	IL-10	IFN-γ
Anti-CD3	-	-	-
Live L.g	+	+	-

Role of the TGF-β1 producing CD4+ CD25+ T cells in the development of disease ?

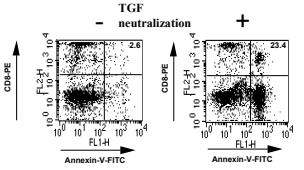


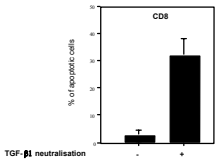


- **TGF-β1 neutralization decreased expression of both CLA and CCR4 but not CD62L and CCR7 on IFN-γ producing cells**

⇒ TGF-β1 implicated in homing of IFN-γ producing CD8+ T cells to skin (anti-parasite function)

Role of the TGF-β1 producing CD4+ CD25+ T cells in the development of disease ?





- **Neutralization of TGF-β1 increased apoptosis in CD8+ T cells**

⇒ TGF-β prevents apoptosis allowing the IFN-γ producing CD8+ T cells to have their effector functions

Conclusions (2)

- **TGF-β1 producing CD4+ CD25+ T cells might be involved in " protective " response against infection with *Leishmania*:**
  - **Inhibit IL-10 production in response to live L.g.**
  - **Maintain surface markers implicated in the homing of IFN-γ producing CD8+ T cells to the skin**
  - **Inhibit apoptosis of CD8+ T cells**

⇒ Allow the IFN-γ producing CD8+ T cells to have/maintain their anti-parasite functions at the local site

CONCLUSION (1)

-in subjects who have never been exposed to *Leishmania*

IFN-γ producing CD8+ T cells specific for LACK
IL-10 producing CD4+ T cells specific for LACK (undetectable in the presence of L.g.)

But amounts of cytokines and % of producing cells vary among subjects

⇒ role of these cells in the resistance /susceptibility to infection with *Leishmania*

⇒ follow-up of naive subjects (soldiers) exposed to Leishmania in the rain forest

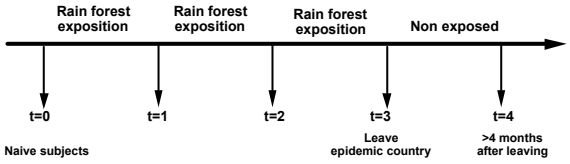
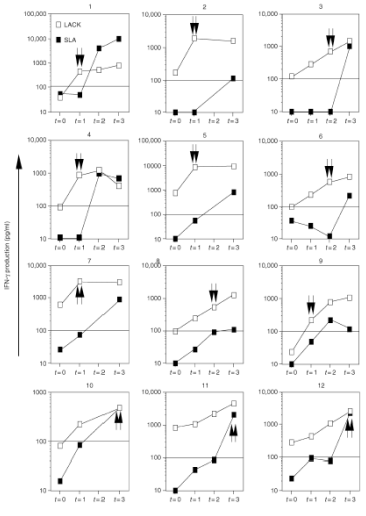
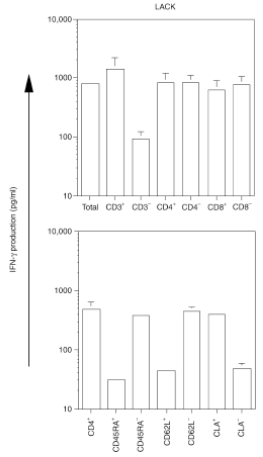


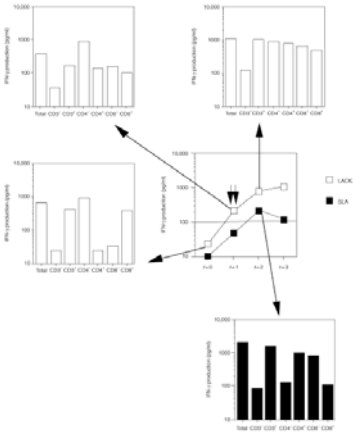
Table 1 Summary of the interferon (IFN)-γ responses to a *Leishmania* homologue of receptors for activated C-kinase (LACK) in subjects exposed to *Leishmania*

	Reactivity against LACK	
	Increased IFN-γ response to LACK†	No increased IFN-γ response to LACK‡
Subjects who became responsive to SLA after exposure to *Leishmania** ($n = 18$)	17	1
Subjects who remained unresponsive to SLA after exposure to *Leishmania** ($n = 161$)	20	141

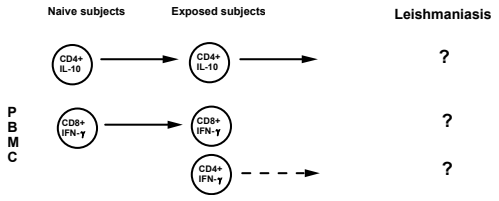
*Positive IFN-γ response to SLA was determined to be >100 pg/ml by analysis 98 healthy subjects without any exposure to *Leishmania*.
†Atleast fivefold increase in IFN-γ response to LACK (as compared with response before exposure to *Leishmania*).
‡Equivalent or decreased IFN-γ production in response to LACK (as compared with response before exposure to *Leishmania*).





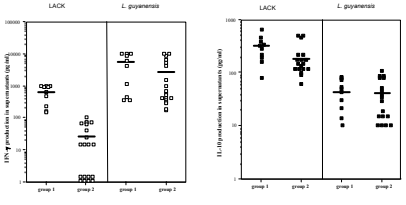


Response to LACK in naïve and exposed subjects



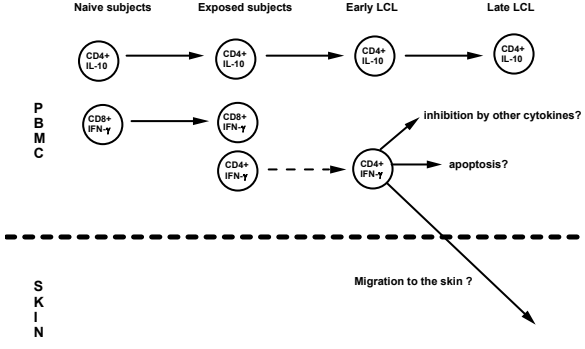
IFN-γ

IL-10

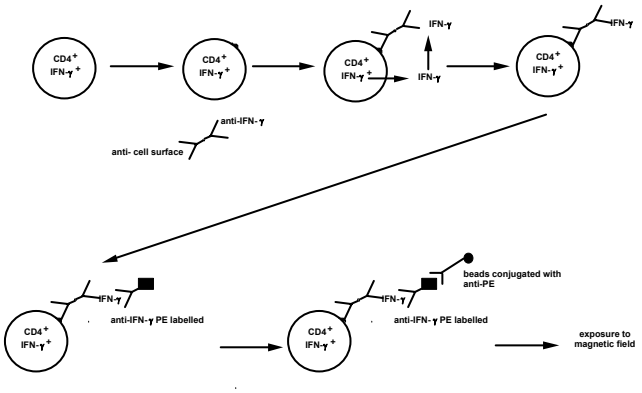


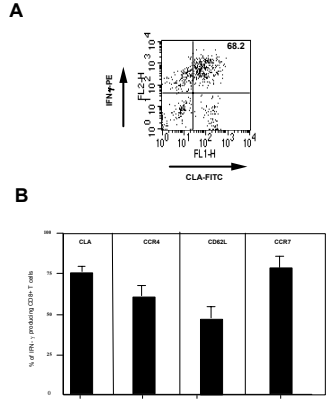
Correlation between IFN-γ production to LACK and clinical and /or parasitological data.

- -number of paraasites per lesion = NO
- -number and location of the lesions = NO
- -presence or absence of adenopathy = NO
- -presence or abscence of lymphangitis = NO
- - duration of the lesions **YES**
  - gpe 1 <30 jours mediane 21 jours
  - gpe 2 > 30 jours medaine 69 jours



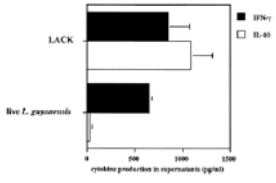
I. Migration to the skin





⇒ **IFN-γ producing CD4⁺ T cells specific to LACK in LCL patients might migrate to skin**

Evidences of the production of suppressive cytokine(s) in response to *L. guyanensis* in PBMC from healthy subjects



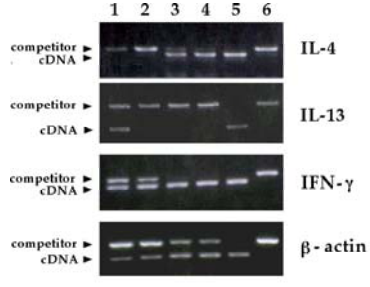
- **IL-10 and IFN-γ producing T cells recognized LACK**
- **Limited IL-10 production in response to live *L. guyanensis***

⇒suppressive cytokines induced by *L. guyanensis* ?

IL-4 and IL-13 not detected

TGF-β?

Analysis of intra lesional cytokines



Detection of intra-lesional cytokines

Definitions

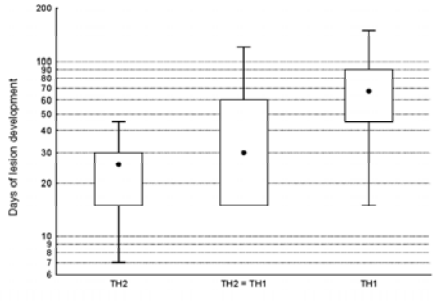
predominance Th2 IL-4 and/or IL-13 > IFN-γ

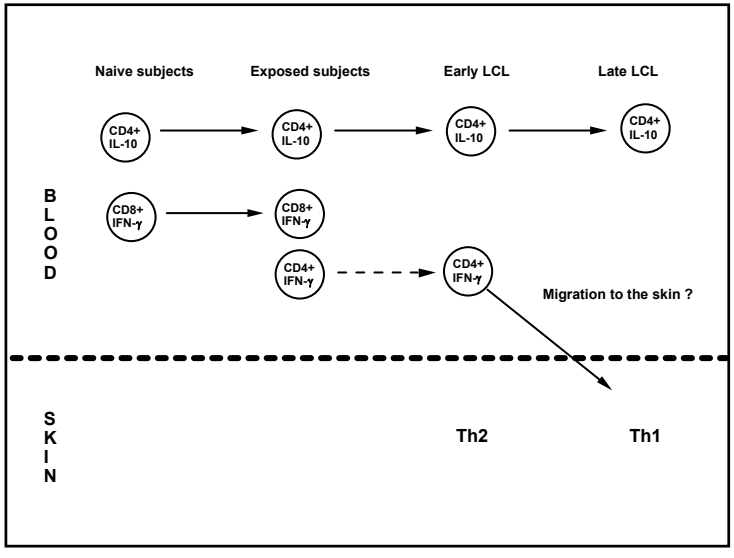
predominance Th1 IFN-γ > IL-4 et IL-13

Results

predominance Th2	39 biopsies
Th2 = Th1	9 biopsies
predominance Th1	28 biopsies

Days of lesion development in Th1 and Th2 lesions

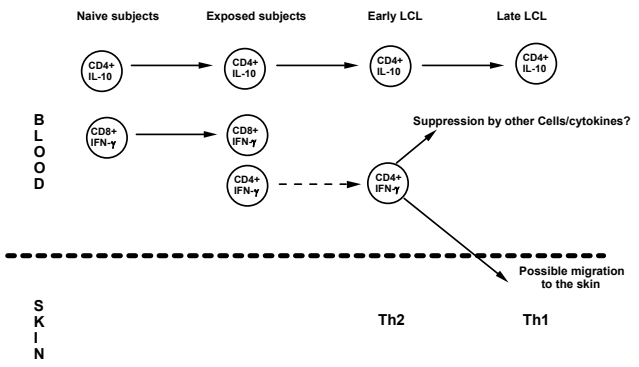




How to prove that LACK specific CD4$^+$ T cells migrate effectively to the skin?

-isolate the cells directly from the lesions
and to analyse their specificity
⇒ but need stimulation of cells

-analyse the TCR repertoire inside the lesions (24 different)
⇒ quantify expression of each Vβ (RT-PCR + ELISA)
⇒ sequencing CDR3
⇒ compare with CDR3 in T cells stimulated with LACK



II. IFN-γ responses to LACK in "late" LCL is due to a particular cytokines

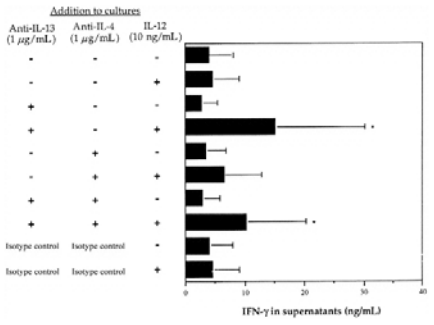
Th2 cytokines known to dow-regulate IFN-γ production

-IL-4 , IL-10 and TGF-β are not detected in supernatants of *Leishmania*-stimulated PBMC from LCL patients.

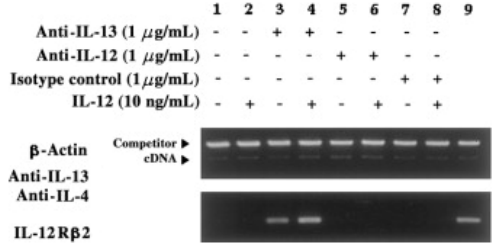
-IL-13 detected in LCL patients

Subjects		IL-13 (pg/ml)	IL-4 (pg/ml)	IFN-γ (pg/ml)
Active patients (n=22)	LCL	159±30**	9±3	5916±1420*
Healed patients (n=5)	LCL	4±4	ND	940±48*
Normal controls (n=10)		12±25	ND	54±27

Neutralisation of IL-13 restore IFN-γ production in response to IL-12



IL-13 dow -regulate IL-12Rβ2 chain on T cells stimulated with *L. guyanensis*



Conclusions (1)

-LACK induces IFN-γ production in PBMC from subjects never exposed to *Leishmania*

-IFN-γ production in response to LACK increase during exposition to *Leishmania*

-IFN-γ production in LCL patients (only during eatrly phase)

⇒ LACK might be a good candidate for vaccination

Conclusions (2)

- LACK induces IL-10 production in PBMC from either
in naïve subjects
in subjects exposed to *Leishmania*
in LCL patients

⇒ deactivation of macrophages
⇒ facilitate development of disease?

BUT others antigens like CPA and CPB (protective in mice) induce IL-10 in human

⇒ new function of IL-10?
(necessary to memory responses?)