INSTITUT PASTEUR

UNIVERSITE PIERRE & MARIE CURIE PARIS 6

The 2nd PSU International Teaching Platform on Tumour Immunology and Immunotherapy

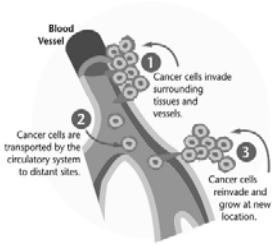
Jointly organized by
Prince of Songkla University, Université Pierre et Marie Curie (Paris 6) and Institut Pasteur

December 15 – 20, 2003
At The Department of Biomedical Sciences
Faculty of Medicine, Prince of Songkla University,
Hat Yai, Songkhla, Thailand

Lecture 3:
Tumor microenvironment
Prof. Hervé Fridman

December 15, 2003

Cancers are capable of spreading through the body by two mechanisms: Invasion and Metastasis



Invasion refers to the direct migration and penetration by cancer cells into neighboring tissues.

Metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body.

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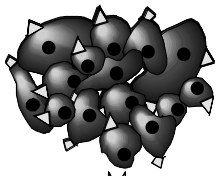
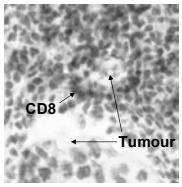
Tumoral micro-environnement



Tumour Ag
- differentiation
- mutated genes
- specific antigen



Micro-environment

Specific T Cells

T Cells
B Cells
Dendritic Cells (mature, immature)
NK Cells
NKT Cells
Macrophages

-> Not Rejected ?

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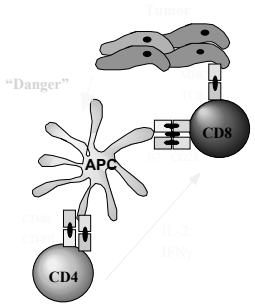
Tumor killing



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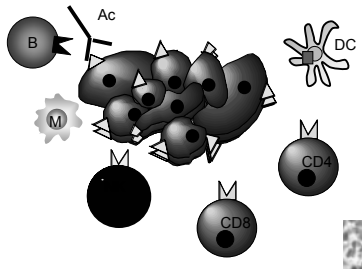
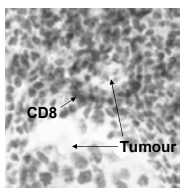


Tumour Ag
- differentiation
- mutated genes
- tumour testis
- viral proteins
...

Tumoral micro-environment



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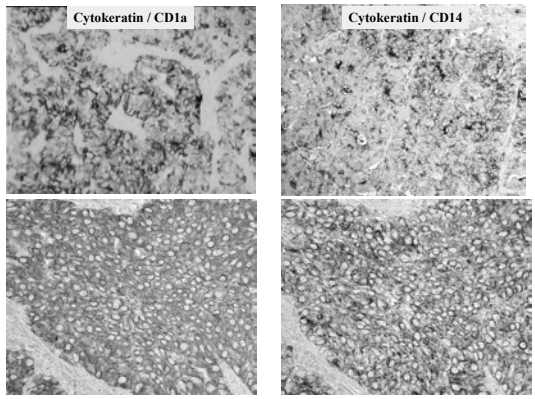
Caractérisation des populations de DC dans l'adénocarcinome



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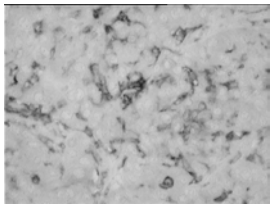
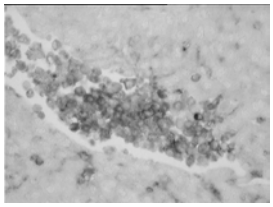
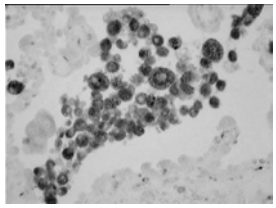
Characterisation of interstital DC and Macrophages

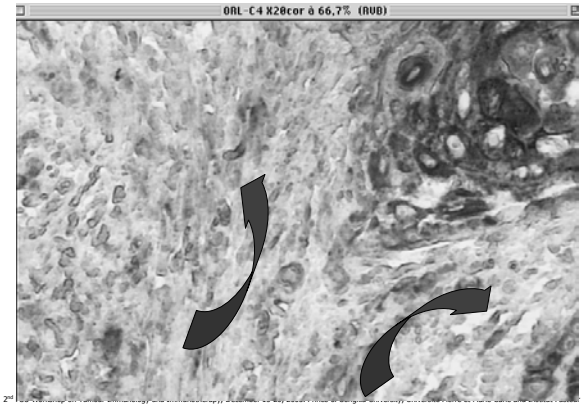
CD14 / CD68



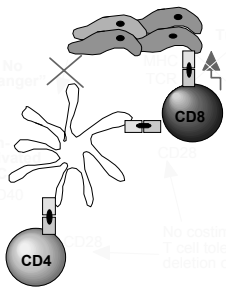




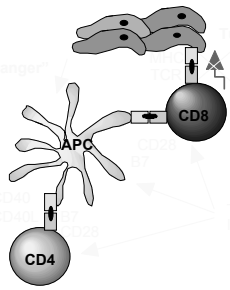
Metastatic lymph node Cytokeratin/CD3



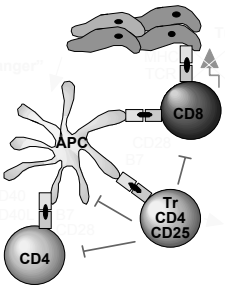
Absence of danger



Tumour-derived suppression



Inhibition by CD4/CD25 regulatory T cells



Multivariate prospective analysis:
234 Patients with head and neck cancers

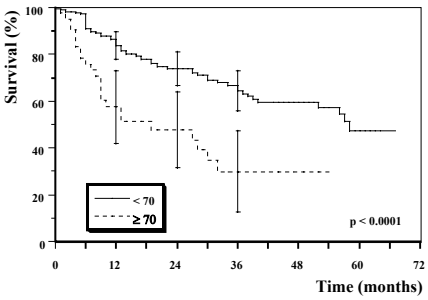
Oral cavity: **112**

Oropharynx: **33**

Hypopharynx: **41**

Larynx: **48**

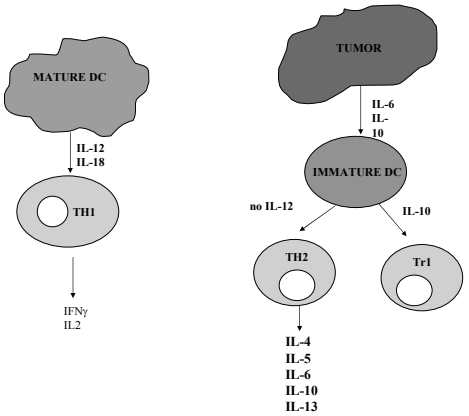
Survival for patients with head and neck squamous cell carcinoma in relation to serum soluble interleukin-2 receptor (sIL-2Rα) levels.



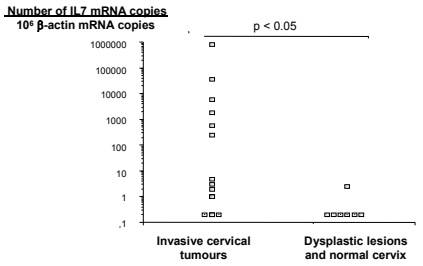
Factors influencing local control, survival and distant metastasis-free interval by the Cox proportional Hazards model.

Order of entry in the model	p value	Relative risk (RR) and 95% CI
Survival		**RR of death**
1. Lymph node involvement	0.0015	
N0N1		1
N2N3		2.3 [1.4 - 3.7]
2. Serum sIL-2R levels	**< 0.0001**	
< 70		**1**
≥ 70		**3.5 [2.1 - 5.9]**
3. Performance status	0.0001	
EZB=0		1
EZB > 0		3.4 [1.9 - 6.1]
4. T stage	< 0.01	
T1 T2		1
T3T4		2 [1.2 - 3.4]
Local control		**RR of local failure**
1. T stage	0.0001	
T1		1
T2T3T4		7.6 [1.8 - 31.8]
2. Lymph node involvement	0.0004	
N0		1
N1N2N3		2.4 [1.5 - 3.8]
3. Performance status	< 0.045	
EZB = 0		1
EZB > 0		1.9 [1- 3.4]
Distant metastasis-free interval		**RR of metastasis**
1. Lymph node involvement	< 0.0001	
N0		1
N1N2N3		6.8 [2.7 – 17.2]
2. Serum sIL-2R levels	**0.0002**	
< 70		**1**
≥ 70		**5.8 [2.4 – 14.3]**

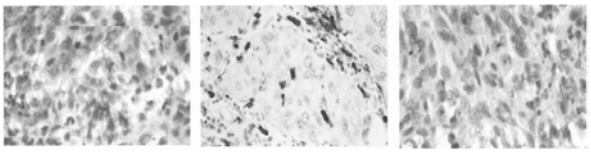
T CELL POLARISATION IN THE TUMOUR MICROENVIRONMENT



Comparative analysis of IL-6 mRNA expression in cervical invasive tumours, dysplatic lesions and normal cervix

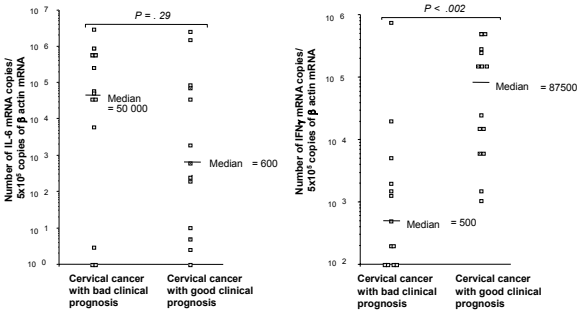


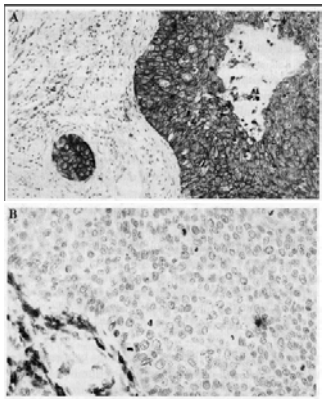
Expression of IL-6 and macrophage infiltration in cervical tumours



IL-6 macrophages Isotype control

Intratumoral expression of IL6 and IFNγ mRNA in patients with non-metastastic cervical cancer





IL-6 AND CANCER

- IL-6 is considered as an autocrine or paracrine growth factor for many tumours (myeloma, lymphoma, renal cell carcinoma, melanoma…)

- High IL-6 serum levels are associated with a poor prognosis and negative clinical outcome in various human cancers.

INTERLEUKIN-17 (IL-17 A)

- First member of an emerging cytokine family (IL-17B, IL-17C IL-17D, IL-17E, IL-17F) expressed as dimers

- Produced by activated memory CD4-T cells

- Pro-inflammatory cytokine which increases the production of chemokines (IL-8, MCP-1, Groα) and hematopoietic growth factor (G-CSF, GM-CSF) thereby promoting the expansion and recruitment of monocytes and neutrophils.

- IL-17 stimulates the production of IL-6 by different epithelial cell lines.

IFN-γ and pronostic value

Loss of IFNγ at the site of tumour → **Bad prognosis**

- Cervical cancer

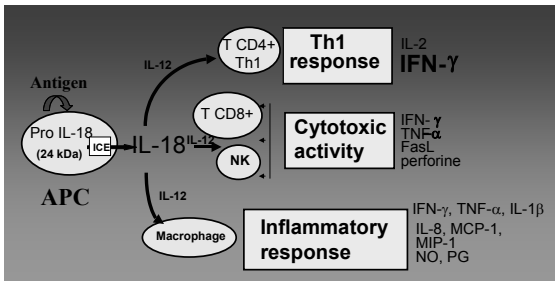
(Tartour E. et al. J. Nat. Cancer Inst. 1998)

- Colon cancer

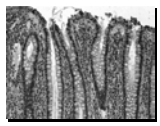
(Pagès F. et al. Int. J. Cancer 1999)

IL-18: an IFN-γ inducing cytokine

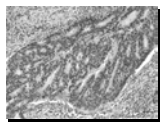
• **IL-18 activity: a multifunctional cytokine**



IL-18 and colon adenocarcinoma



IL-18 is synthesized by epithelial cells of the normal colon mucosa facing the intestinal lumen



• **IL-18 expression decreased / abolished in colon adenocarcinoma**

IL-18 and colon adenocarcinoma

• **Clinical outcome :**

- 5/7 patients with markers of IL-18 activity (FasL, IFN-γ) had a cancer strictly confined to the colon.

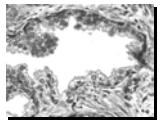
- 7/7 patients with no marker of IL-18 activity (FasL, IFN-γ) presented with distant metastases.

Production of active IL-18 at tumour site may be involved in the host anti-tumour immune defence

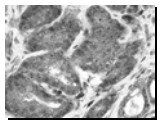
IL-18 and prostate cancer

• 36 cases of clinically localized prostate cancer studied

- 27/36 cases with tumour cells producing IL-18
- heterogeneous tumour IL-18 expression (<10 to >66% IL-18$^+$ cells)
- not correlated with the pathological stage or the Gleason score



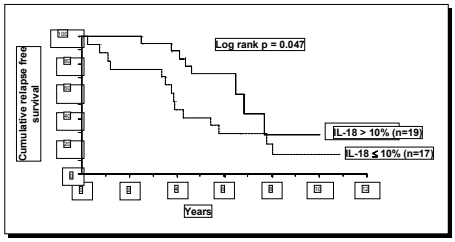
Normal prostate
basal cells express IL-18



Prostate cancer
Tumour cells express IL-18

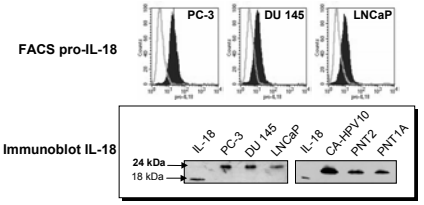
IL-18 and prostate cancer

• median follow-up: 95 months (75-115)
• clinical outcome: 12 disease-free patients
22 relapses (11 bone metastasis)



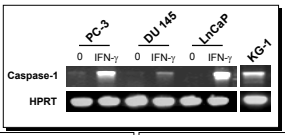
IL-18 and prostate cancer

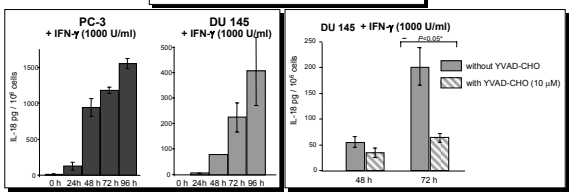
• Prostate tumour cell lines synthesize the immature form of IL-18 (24 kDa)



IL-18 and prostate cancer

• IFN-γ induces caspase-1 mRNAs and IL-18 secretion





Interleukin-18 and the EBV pos. lymphomas

• The EBV virus:

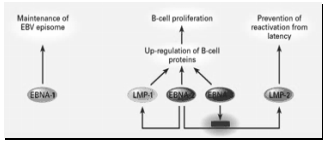
- A human gamma herpes virus

- Largely B lymphotropic (viral gp350 - CD21)

- Associated diseases:
  - Infectious mononucleosis
  - Nasopharyngeal carcinoma
  - Burkitt's lymphoma
  - Hodgkin's disease
  - B-Lymphoproliferative disease in the immunocompromised host

Interleukin-18 and the EBV pos. lymphomas

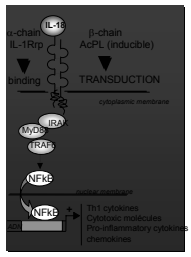
• EBV latent genes



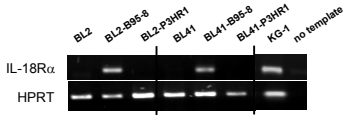
Principal disease associations of EBV	EBNA-1	EBNA-2	EBNA-3	LMP-1	LMP-2	EBER	latency
B lymphoproliferative disease in the immunocompromised host	+	+	+	+	+	+	Type 3

Interleukin-18 and the EBV pos. lymphomas

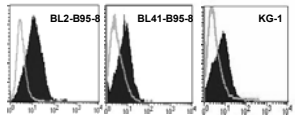
• IL-18Rα



IL-18Rα RT-PCR :

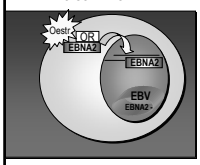


FACS analysis of IL-18Rα

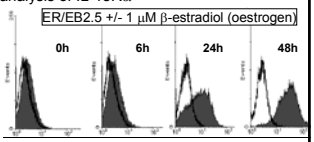


Interleukin-18 and the EBV pos. lymphomas

ER/EB2-5 cell line:

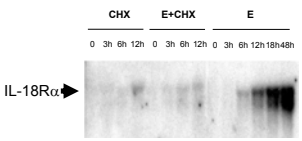


FACS analysis of IL-18Rα



Northern Blot of IL-18Rα

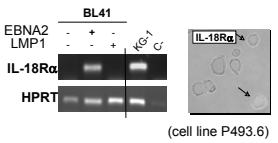
ER/EB2.5 +/- oestrogen (E) +/- cycloheximide (CHX)



Interleukin-18 and the EBV pos. lymphomas

• The mechanism is indirect and does not require other viral proteins

IL-18Rα RT-PCR
IL-18Rα immunofluorescence study:
BL41 transfected with LMP1 ou EBNA2 cDNA

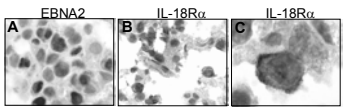


(cell line P493.6)

- c-myc overexpression does not induce IL-18Rα

Interleukin-18 and the EBV pos. lymphomas

Immunohistochimical study of EBNA2 and IL-18Rα

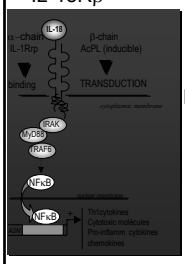


Diffuse large B-cell lymphoma (DLBCL)

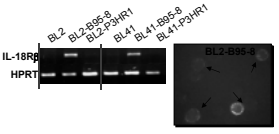
Patient No.	Histology	anatomic site	EBV status (EBER)	EBV status EBNA2	IL-18Rα
1	DLBCL (I-BL)	brain	+	-	-
2	DLBCL (IB)	brain	+	+ (73 %)	+ (51 %)
3	DLBCL (IB)	brain	+	+ (68 %)	+ (20 %)
4	DLBCL (IB)	brain	+	+ (66 %)	+ (58 %)
5	DLBCL (IB)	brain	+	+ (71 %)	+ (11 %)
6	DLBCL (IB)	brain	?	-	+ (66 %)
7	DLBCL (IB)	lymphe node	+	-	-
8	DLBCL (IB)	testis	-	-	-
9	DLBCL (IB)	skin	-	-	-
10	DLBCL (CB)	lymphe node	-	-	-
11	DLBCL (IB)	lymphe node	-	-	-
12	DLBCL (IB)	lymphe node	+	+ (34 %)	-
13	DLBCL (IB)	ND	+	-	-

Interleukin-18 and the EBV pos. lymphomas

• IL-18Rβ

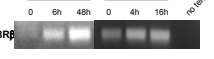


RT-PCR and Immunofluorescence studies of IL-18Rβ

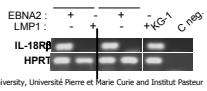


- induction of IL-18Rβ by EBNA2

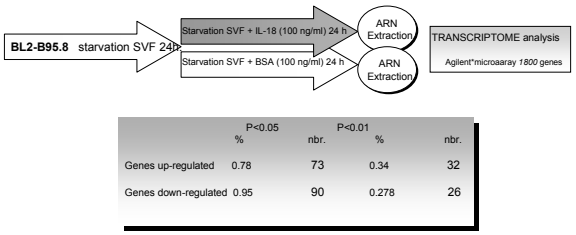
IL-18Rβ RT-PCR
ER/EB2.5 +/- β-estradiol



IL-18Rβ RT-PCR
BL2, BL41+/- transfected by EBNA2 and LMP1 cDNA

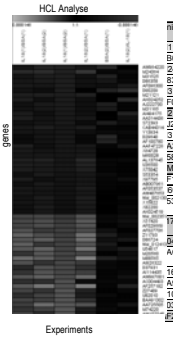


Interleukin-18 and the EBV pos. lymphomas



	P<0.05 %	P<0.05 nbr.	P<0.01 %	P<0.01 nbr.
Genes up-regulated	0.78	73	0.34	32
Genes down-regulated	0.95	90	0.278	26

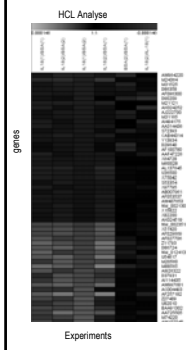
Interleukin-18 and the EBV pos. lymphomas



genes up-regulated by IL-18

UniqID	nom	Diff. IL-18/duplicate
11124	major histocompatibility complex, class II, DQ alpha 1	-0.62657
B032994	p53 inducible protein	-0.535907
24364	major histocompatibility complex, class II, DQ beta 1	-0.531635
83582	major histocompatibility complex, class II, DQ beta 1	-0.530692
31525	major histocompatibility complex, class II, DN alpha	-0.5199315
F096300	mitogen-activated protein kinase kinase kinase kinase 4	-0.505963995
21121	small inducible cytokine A5 (RANTES)	-0.482886
J222700	transforming growth factor beta-stimulated protein TSC-22	-0.465081
31165	tumor necrosis factor, alpha-induced protein 6	-0.460434
A314436	chaperonin containing TCP1, subunit 7 (eta)	-0.435717
58964	regulatory factor X, 1 (influences HLA class II expression)	-0.41248045
M_004233	CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	-0.40771
F100780	WNT1 inducible signaling pathway protein 2	-0.401959
60028	major histocompatibility complex, class II, DQ beta 1	-0.389958
53354	CD83 : B-cell activation protein=B-G antigen IgV domain homolog [Human, SAC-activated B lymphocytes, Genomic/mRNA, 2574 nt].	-0.351445
17418	CD35 : CR1, Human complement receptor type 1 (alleles S and F) gene, exon 47 and complete cds's.	-0.30322
04343	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	-0.271672
AC51348	"homologous to members of the I-kappa B family; protein binds NF-kappa B proteins"	-0.269418
16961	tumor protein 63 kDa with strong homology to p53	-0.24275
A903016	"putative chemokine receptor; GTP-binding protein"	-0.232904
10202	platelet-activating factor receptor	-0.2289938
03066	major histocompatibility complex, class II, DO beta	-0.22256
F240467	toll-like receptor 7	-0.21287223

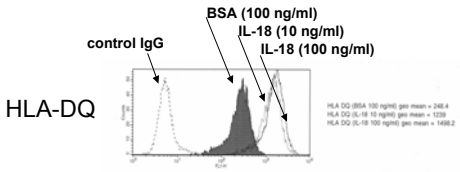
Interleukin-18 and the EBV pos. lymphomas



genes down-regulated by IL-18

UniqID	nom	Diff. IL-18/duplicate
Y07921	protease, serine, 11 (IGF binding)	0.2343
L19593	CXCR2 : interleukin 8 receptor, beta	0.237909
NM_006022	transforming growth factor beta-stimulated protein TSC-22	0.238839998
X68742	integrin, alpha 1	0.239028
M26038	major histocompatibility complex, class II, DR beta 5	0.251979995
X02544	orosomucoid 1	0.297884
U66061	protease, serine, 1 (trypsin 1)	0.332468
M20560	annexin A3	0.338102
X72875	B-factor, properdin	0.33859
Y16645	MCP2 : small inducible cytokine subfamily A (Cys-Cys), member 8 (monocyte chemotactic protein 2)	0.408162
Z27409	EphA1	0.4123799
AA725505	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	0.4229
M74220	plasminogen	0.430639
AF005213	ankyrin 1, erythrocytic	0.435001
AW157548	insulin-like growth factor binding protein 5	0.437501
M57853	protein S (alpha)	0.44647
M76180	dopa decarboxylase (aromatic L-amino acid decarboxylase)	0.455848995
S65125	AXL receptor tyrosine kinase	0.465520998
AF016917	gamma-aminobutyric acid (GABA) A receptor, delta	0.47349
AI041851	cathepsin L	0.473559
AJ001531	protease, serine, 12 (neurotrypsin, motopsin)	0.488375
N31450	glucocorticoid receptor DNA binding factor 1	0.496001
AA310902	CD3D antigen, delta polypeptide (TiT3 complex)	0.504099998
M69199	putative lymphocyte G0/G1 switch gene	0.505545
U16997	RAR-related orphan receptor C	0.55893
AW950033	apolipoprotein C-II	0.5910826
AF004877	collagen, type I, alpha 2	0.600655

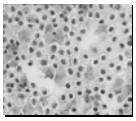
Interleukin-18 and the EBV pos. lymphomas



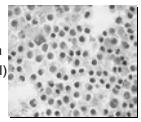
Interleukin-18 and the EBV pos. lymphomas

Immunohistochemistry: EBNA2

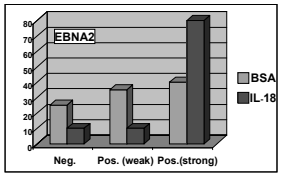
BL2-B95.8
Non-stimulated



BL2-B95.8
stimulated with
IL-18 (100 ng/ml)
96 hours



strong nuclear staining



Interleukin-18 and tumours - Conclusions

- IL-18 is (also) a cytokine of the epithelia, that plays an important role in the lesions of Crohn's disease
- IL-18 is detected in a large number of human tumours and could be marker of good prognosis
- Multiple mechanisms probably account for the IL-18-antitumour activity However, IL-18 has been reported to be detrimental in some tumours such as melanoma
- The interferons modulates the secretion of IL-18 of tumours producing the cytokine
- In EBV-positive lymphomas, EBNA2 induces a functional IL-18R. IL-18 could modulate the expression level of the viral latency proteins and the MHC-class II molecules