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 4:
Cytokines and Cancer
Prof. Eric Tartour

December 16, 2003

CYTOKINES AND CANCER

1 Pro-tumor activity of cytokines

- Growth or anti-apoptotic factors for tumour cells.
- Inhibitors of T cell activation (TGFβ , IL-10)
- Inhibitors of dendritic cell maturation (IL-6, IL-10...).

2 Anti-tumor activity of cytokines

. Bias in TH1 polarisation (low concentrations of TH1 cytokines, ↗RsIL-2…)
. Clinical use of cytokines in immunotherapy.

3 Dual activity of cytokines in the control of tumor growth.

- IL-17 a model of cytokine with ambivalent activity in oncology.
- Other examples of cytokines with dual activity
- Mechanisms underlying these pleiotropic and antagonist activities.

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Cytokines as growth factors for tumour cells

Cytokines stimulate the growth of many tumour cell lines.

- GM-CSF is a growth factor for human ovarian cancer cells
- IL-1 can promote the growth of leukemia cells by inducing GM-CSF
- Cytokines may act as autocrine growth factors or paracrine growth factors.

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Interleukin -6

- IL-6 is a growth factor for AIDS Kaposi 's sarcoma derived cells, myeloma, certain T and B cell lymphoma, renal cell carcinoma, hepatocarcinoma, cervical carcinoma and prostate carcinoma cell line.

- Recent studies have shown that IL-6 is a survival factor for myeloma cells by inducing Mcl-1 (Jourdan M. Cell Death Diff 2000)

- Anti IL-6 mAb therapy in myeloma patients : Inhibition of IL-6 activity without real impact on clinical parameters.

- Since IL-6 has been linked with drug resistance mechanisms, association of anti-IL-6 with chemotherapy is being tested.

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Cytokines (IL-10, TGFβ) as immunosuppressive factors with the ability to inhibit T cell activation

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TGFβ

. Drives the balance to TH2 via IL-10 as an intermediate
. Inhibits TH1-type responses directly
. Inhibits T cell activation

IL-10

. Downmodulates the expression of TH1 cytokines
. Regulates expression of TGF-β type II receptor.
. Decreases the expression of MHC molecules
. Inhibits the differentiation of dendritic cells.

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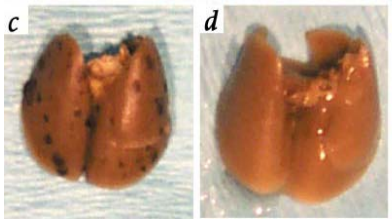
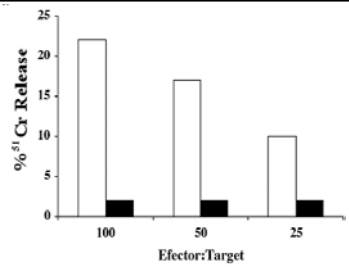
Wild Type Mice DN TGFRII Mice



2×10^5 B16-F10 i.v.

Blockade of TGF- signaling in T cells renders mice resistant to tumor challenge.

Gorelik L. Nature Med 2001



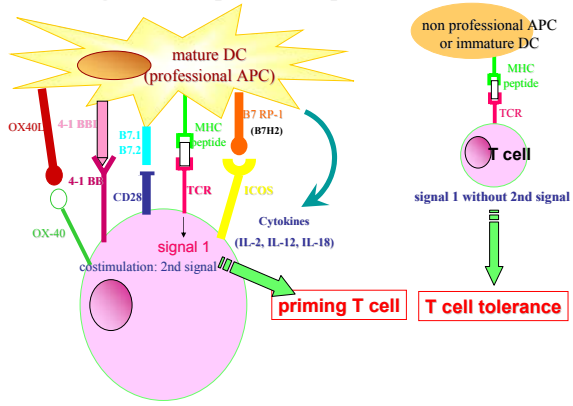
In vivo generation of tumor-specific CD8+ CTLs in the absence of TGFβ- signaling in T cells.

Spleen cells were isolated from transgene-positive (□) or transgene-negative (■) littermate mice challenged with 10^6 live EL-4 cells 10 days prior and were evaluated for their lytic activity by the 51Cr release assay against EL-4 targets.

Gorelik L Nature Med 2001

Cytokines inhibit the maturation of dendritic cells during tumour progression

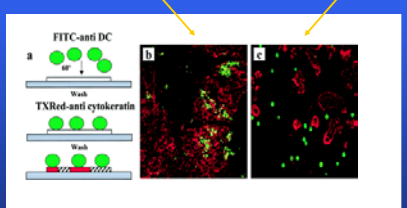
Two signal are required for optimal T cell activation



Only immature dendritic cells are found in contact with tumour cells

Immature DC (green) bind to tumour cells (red)

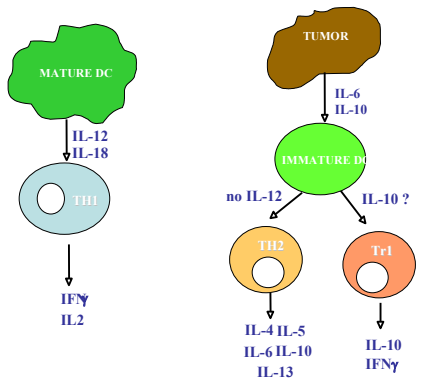
Mature DC (green) don't bind to tumour cells (red)



(Bell et al 2000)

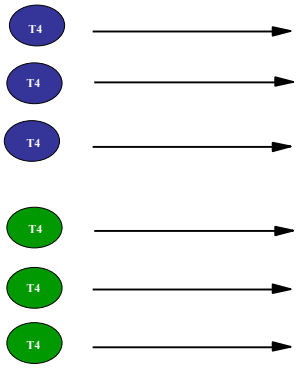
Mature DC were virtually absent in liver tissue from patients with hepatocellular carcinoma (Chen S et al. 2000)

T CELL POLARISATION IN THE TUMOUR MICROENVIRONMENT
ROLE OF CYTOKINES AND STATE OF MATURATION OF DENDRITIC CELLS



Anti-tumour activity of TH1 cytokines

TH1 AND TH2 CLONES (T. MOSMANN)



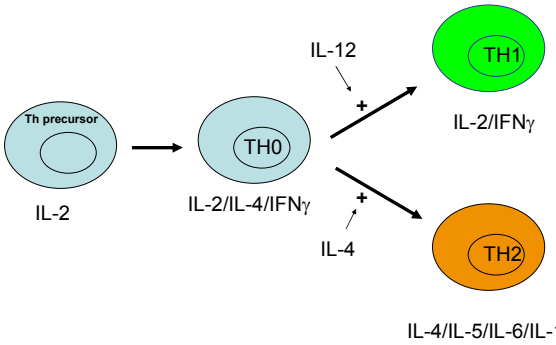
IL-2/IFNγ/TNFβ

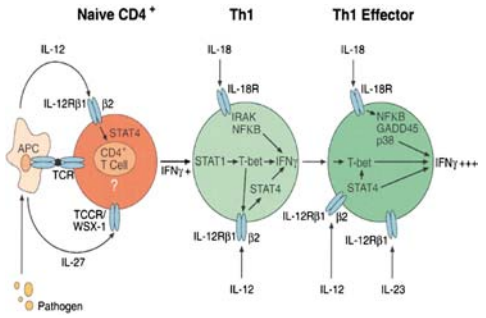
TH1

IL-4/IL-5/IL-6/IL-10/IL-13

TH2

Regulation of CD4 T-cell differentiation





Checkpoint in TH1 development

- The receptor for IL-18 is induced by IL-12
- T bet induced IL-12 Rβ2 expression

ROLE of TH1 and TH2 cytokines

CD4-TH1
IL-2, IFN γ, TNFβ →

- Stimulation of T lymphocytes (Cell mediated immunity)
- Activation of macrophages cytotoxicity
- Favour IgG2a antibody switch.

CD4-TH2
IL-4, IL-5, IL-6, IL-10 →

- Stimulation of B lymphocytes (humoral immunity)
- Favour IgE and IgG1 antibody switch (IgG 4 in human)
- Activation of mastocytes and differentiation of eosinophils (Allergy).

Bias in TH1 polarization in cancer

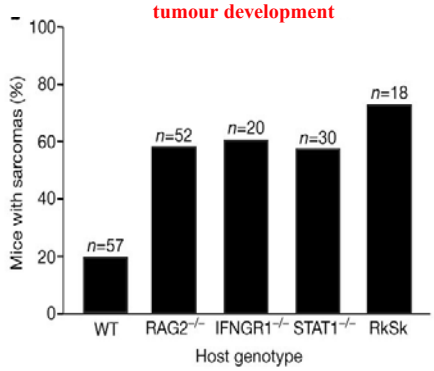
Low TH1 cytokine concentrations in cancer, ↗ RsIL-2

LYMPHOKINE PROFILE OF SPLENIC T CELLS FROM NORMAL AND RENAL CELL CARCINOMA BEARING MICE

Source of Splenic T cells	INTERFERON γ (U/ml)	INTERLEUKIN 2 (Pg/ml)	INTERLEUKIN 4 (Pg/ml)
Normal mice	3.4	965	< 5
Tumour-Bearing mice	< 1	460	40

Ghosch J. Natl Cancer Inst. 1995

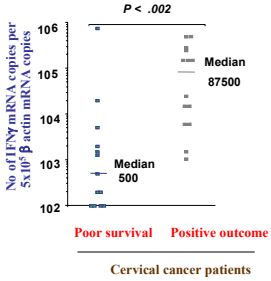
Lymphocyte-deficient mice are highly susceptible to MCA-induced tumour development



Shankaran et al. Nature 2001

Prognostic value of cytokines in cervical carcinomas

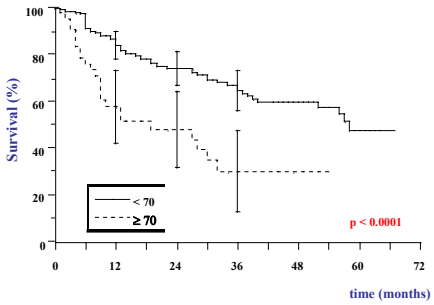
Low levels of IFNγ mRNA in poor prognosis cervical carcinoma patients

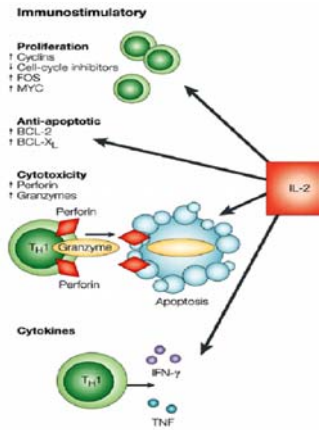


SWITCH IN THE PATTERN OF CYTOKINE FROM TH1 (IL2, IFNγ) to TH2 (IL-4, IL-6, IL-10) GROUPS IN CANCER PATIENTS

- IL-2 and IFNγ in TIL derived from breast and renal cell carcinomas
- IL-6 in invasive cervical carcinomas, bladder and renal cell carcinomas
- IL-10 in melanoma, glioma, bronchogenic and renal carcinomas

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





Therapeutic use of cytokines in oncology

IL-2: Response rate of 10-20% with approximatively 5% complete response in renal cell carcinoma and melanomas.

Initially proposed as high-dose intravenous bolus or intermediate dose-continuous infusion but these schedules give rise to toxicity: capillary leak syndrome

(Rosenberg SA N Eng J Med 1987; West WH. N Eng J Med 1987)

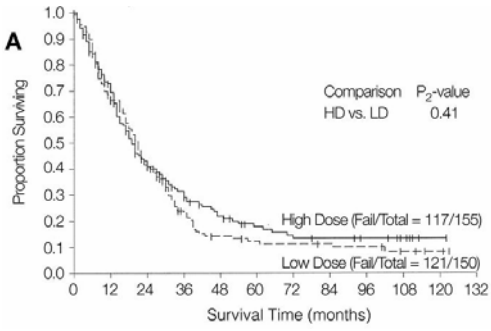
	High-Dose	Low-Dose	Subcutaneous
Total courses (100%)	285	272	181
Thrombocytopenia	9.2	1.5	0
Hyperbilirubinemia	3.2	0.7	0
ALT	3.2	0.7	0.6
Nausea/vomiting	13.4	8.5	3.3
Diarrhea	9.2	3.7	1.7
Peripheral edema	0.4	2.6	0
Creatinine (≥ 8.0)	1.1	2.6	0.6
Oliguria (≤ 80 mL/8 h)	12.0	7.7	1.1
Pulmonary	4.2	1.1	0
Malaise	20.5	9.9	9.4
Infection	2.8	2.6	1.1
Arrhythmia, atrial	4.2	1.5	0
Hypotension	36.4	2.9	0
CNS level of consciousness	2.5	2.6	0
CNS orientation	10.2	3.7	1.7
Death	0	0	0

Toxicities of all patients receiving interleukin-2 therapy for renal cancer - Percentage of courses with grade 3 or 4 toxicity

Yang JC. J Clin Oncol 2003

- To improve tolerance, several investigators proposed subcutaneous administration, which yielded efficacy comparable to that of intravenous administration with the added benefit of less toxicity and outpatient treatment (Buter J . Semin Oncol 1993; Lopez-Hanninen E. J Urol 1996)

- However it persists some controversies about the equivalence of high-dose intravenous IL-2 with low dose regimen



Overall survival of patients randomly assigned to either low-dose (LD) or high dose (HD) intravenous (IV) bolus interleukin-2

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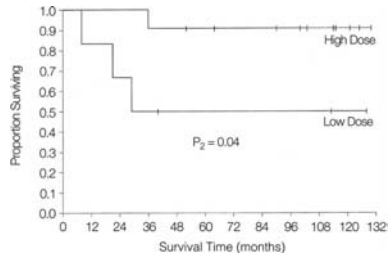
Duration (months)					
High-Dose IV IL-2		Low-Dose IV IL-2		Subcutaneous IL-2	
CR	PR	CR	PR	CR	PR
130+	37	128+	24	78+	28
121+	28	113+	23	13	28
115+	24	40+	22		17
114+	23	20	21+		15
103+	19	19	15		9
100+	17	3	13+		8
90+	17		11		2
52+	16		11		
45	15		8+		
23	14		7		
19	14		7		
	14+		4		
	13		4		
	10				
	9				
	8+				
	8				
	8				
	7				
	6				
	4				
	4				

NOTE. Bold values are for patients concurrently randomly assigned between three arms. + indicates response is ongoing.
Abbreviations: IV, intravenous; IL-2, interleukin-2; CR, complete response; PR, partial response.

Response durations

(Atkins J Clin Oncol 1999; McDermott D ProcASCO 2001) Yang JC. J Clin Oncol 2003



Survival of patients completely responding to high-dose versus low-dose intravenous interleukin-2

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Combination of IL-2 with IFNα or chemotherapy

In a randomized study comparing continuous infusion IL-2 in monotherapy or subcutaneous IFNα monotherapy or a combination of intravenous IL-2/subcutaneous IFNα, the author reported a significant superiority in terms of response for the combination regimen, but this superiority was without survival benefit. (Negrier S. N Eng J Med 1998)

Addition of chemotherapy increased the response rate and toxicity without real impact on survival (Tourani JM J Clin Oncol 1998).

Since IL-2 benefits to only a subgroups of cancer patients many attempts have been pursued to identify predictive factors for response

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Sites of Metastases Versus Response

Site of Metastases	No. of Patients	Responders No.	Responders %	P_2
SQ and/or cutaneous alone				
Yes	28	15	53.6	.000001*
No	346	43	12.4	
SQ/cutaneous + LN alone				
Yes	23	5	27.7	.38
No	351	53	15.1	
LN alone				
Yes	29	6	20.7	.42
No	345	52	15.1	
Visceral alone				
Yes	69	8	11.6	.32
No	305	50	16.4	
Visceral + SQ/cutaneous alone				
Yes	61	9	14.8	.86
No	313	49	15.7	
Visceral + LN alone				
Yes	56	5	8.9	.14
No	318	53	16.7	
Bone + any other site(s)				
Yes	30	5	16.7	.80
No	344	53	15.4	
Brain + any other site(s)				
Yes	21	1	0.05	.056
No	353	57	16.1	

*Odds ratio, 8.13; 95% confidence interval, 3.33 to 19.81.
Abbreviations: SQ, subcutaneous; LN, lymph node(s).

Phan GQ J Clin Oncol 2001

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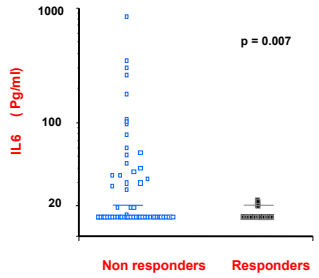
Melanoma patients with high serum levels of IL-6 before therapy were unlikely to respond to IL-2 therapy.



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Posttreatment Factors Versus Response and the Incidence of Long-Term Immunologic Side Effects of IL-2 in Responders

	No. of Patients	Responders No.	Responders %	Odds Ratio	95% CI	P_2
TSH						
Normal	153*	15	9.8	2.25	1.16-4.54	.01
Abnormal	219*	43	19.6			
FT4						
Normal	172*	17	9.9	2.35	1.24-4.60	.0049
Abnormal	200*	41	20.5			
Vitiligo						
Present	84	28	33.3	4.33	2.29-8.14	$< 10^{-6}$
Absent	290	30	10.3			

	Abnormal TSH No.	Abnormal TSH %	Abnormal FT4 No.	Abnormal FT4 %	Vitiligo No.	Vitiligo %
Responders	43/58	74.1	41/58	70.7	28/58	48.3
Nonresponders	176/314	56.1	159/314	50.6	56/316	17.7

Abbreviation: CI, confidence interval.
*The total number of assessable patients was 372 because two nonresponders did not have TSH/FT4 levels during follow-up.

Phan GQ J Clin Oncol 2001

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IFNα

-Upregulation of MHC class I and II expression

-Potentiation of effector T- and natural killer (NK cells)

-Maturation of dendritic cells

-Antiangiogenic effect

-Direct inhibition of tumour growth.

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IFNα

Indications:

Hairy cell leukemia, chronic myelogenous leukemia, Kaposi sarcoma associated with VIH, Follicular lymphoma

- A randomized study showed an improvement in survival in patients with metatstatic renal cell carcinoma treated with IFNα (Lancet 1999)

- Low dose IFNα monotherapy has failed to provide significant clinical improvement in patients at high risk for melanoma (Cascinelli N Lancet 2001; Pehamberger H J Clin Oncol 1998)

Side effects

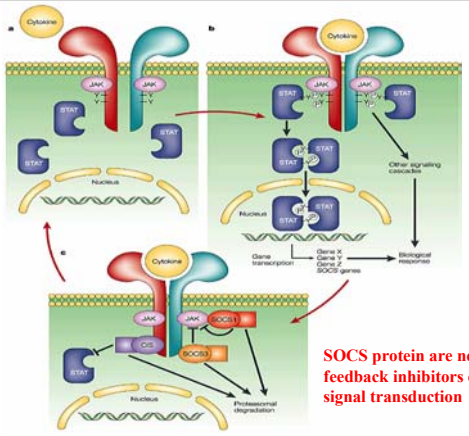
-Flu-like syndrom

-Depression

- Nausea, vomiting, diarrhea

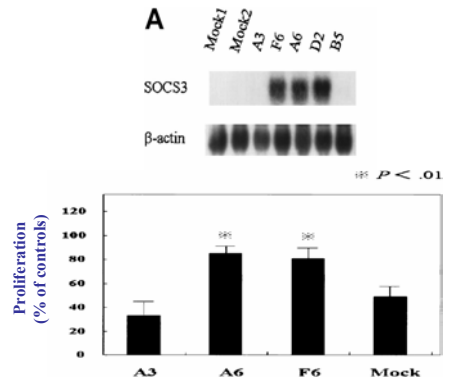
- Increase of ALT

- Retinopathy

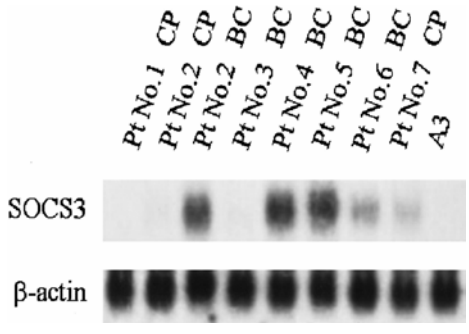


SOCS protein are negative feedback inhibitors of cytokine signal transduction

SOCS 3 confers resistance to IFNα in CML cells



Sakai Blood 2002



Expression of SOCS 3 mRNA in fresh CML cells from chronic phase (CP) or blastic crisis (BC) patients

IL-12

- Development of Type 1 T-cell response

- Enhanced recruitment and activation of NK cells

- Antiangiogenic effect

Toxicity

A phase II trial led to a fatal outcome in two renal carcinoma patients which has markedly delayed clinical testing

Modification of the administration schedule with a pre-dose has reduced the toxicity.

- Clinical results were rather disappointing in solid tumours (melanoma, renal cell carcinoma, ovarian cancer, colon cancer)

- Better results were reported in a small cohort of patients with cutaneous T- cell lymphoma and Sezary syndrome where 56% response rate was observed including tumour reduction (Rook AH 2001)
This was not entirely unexpected since these tumour cells are highly skewed to a TH2 phenotype.

Dual activity of cytokines in the control of tumour growth

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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.

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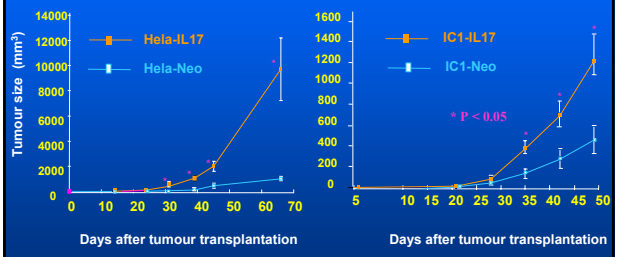
IL-17 increases the growth rate of human cervical tumours transplanted in nude mice



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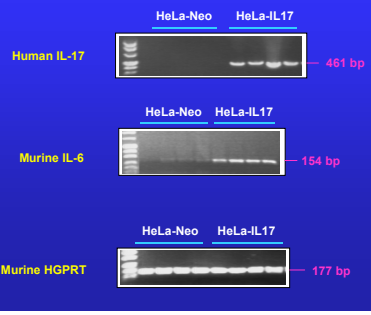
Increased murine IL-6 mRNA expression in biopsies derived from IL-17-transfected HeLa cells transplanted in nude mice.



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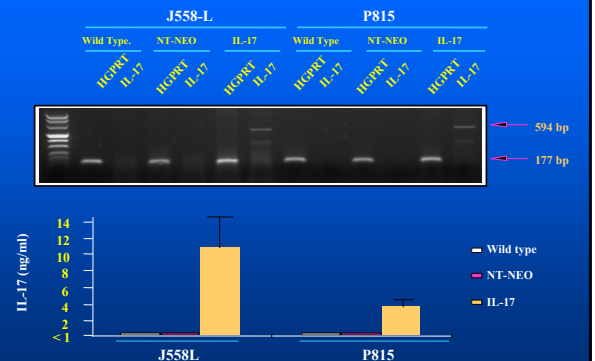
Characterization of mIL-17-transfected P815 and J558L cell lines



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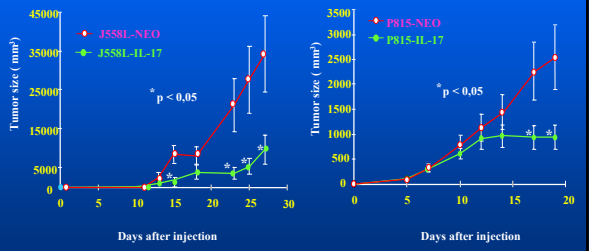
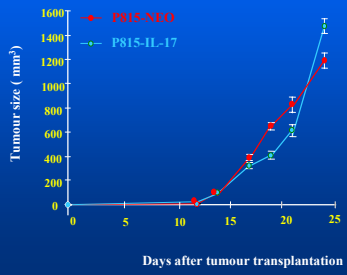
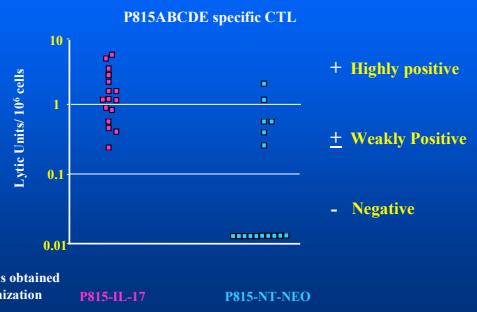
IL-17 inhibits tumor growth in immunocompetent mice



IL-17 does not inhibit the growth of the P815 mastocytoma in nude mice.



IL-17 increases the generation of P815-specific CTL

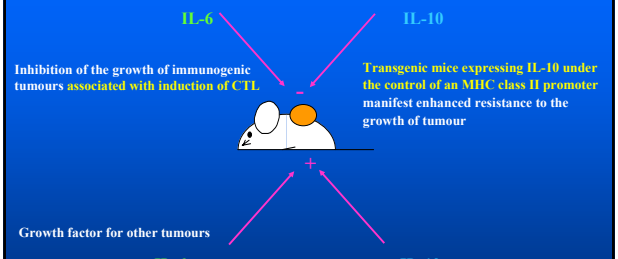


IL-17 a two faces cytokine

- In non immunogenic tumours or in the absence of T lymphocytes, IL-17 promotes tumour growth: this effect seems in part mediated by IL-6.

- In contrast, IL-17 inhibits the growth of immunogenic tumours by means of a T cell dependent mechanism.

This ambivalent activity in the control of tumour growth has also been observed for other cytokines



Factors regulating cytokine activities in cancer patients

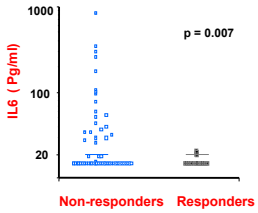
Stage and differentiation of the tumours

- IL-6 inhibits *in vitro* primary melanoma cell proliferation, whereas it rather acts as a growth factor for metastatic melanomas (Kerbel et al. Oncogene 1999)

- TGFβ switch from tumour suppressor to prometastatic factor during breast cancer progression (Tang B J Clin Invest 2003)

- Production of cytokines by tumour cells often reflects a progression of the disease and is associated with loss of sensitivity of tumour cells to the inhibitory activity of cytokines (IL-6, TNFα...).

- Melanoma patients with high serum levels of IL-6 before therapy were unlikely to respond to IL-2 therapy. (Blay et al, Tartour et al…)



Dose and schedule of administration

IL-2: well known as an anti-tumour cytokine. However …
At high doses or if it was administered too frequently, the anti-tumour activity of IL-2 is abolished. (Schmidt W PNAS 1995). This paradoxical effect may be mediated by the induction of CTL apoptosis (Schrikant P J Immunol 2002)

IL-12: inhibits tumour growth in a dose dependent manner but leads to the development of an antitumour immune response when IL-12 is expressed at the tumour site at the relatively small amount (Tahara H Cancer Res 1994)

How may we improve the clinical use of cytokines in cancer patients

- Better selection of cancer patients to be included in therapy by cytokines (tumour stage, presence and site of metastases)

- Inflammatory syndrome (CRP, IL-6…): high risk of resistance to immunotherapy.

- Development of pharmaco-immunological study to better design the dose and schedule of administration of cytokines.

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Benchetrit F
Ciree A
Gey A
Sautes-Fridman C
Fridman WH

Schering Plough
Fossiez F
Lebecque S

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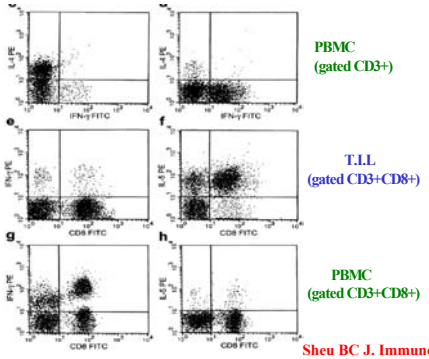
Nagarajan B
Arivu Sambandan

Predominant Th2/Tc2 Polarity of Tumor-Infiltrating Lymphocytes in Human Cervical Cancer



Sheu BC J. Immunol 2001