Immunology for the Non-immunologist and Measuring immunity in patients

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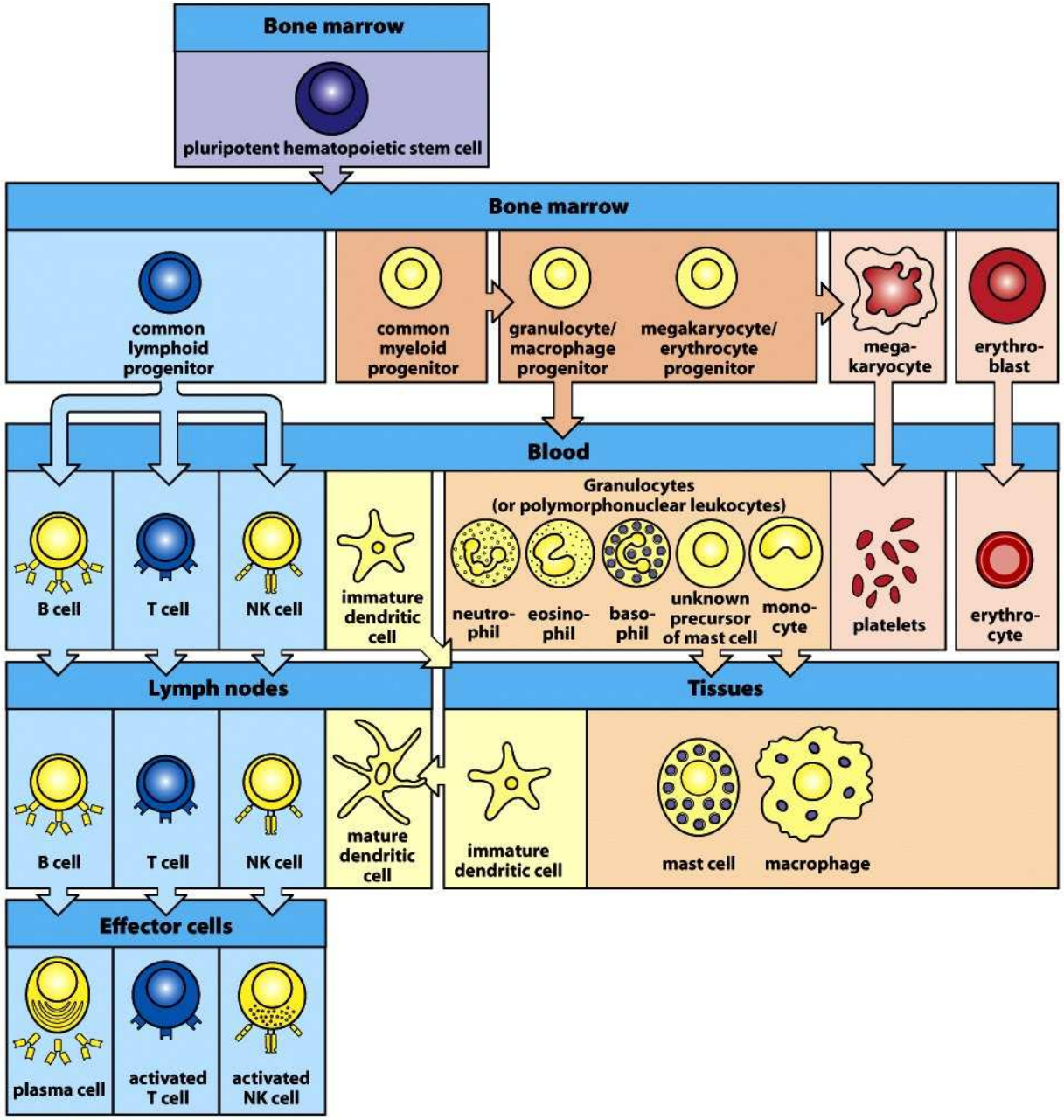
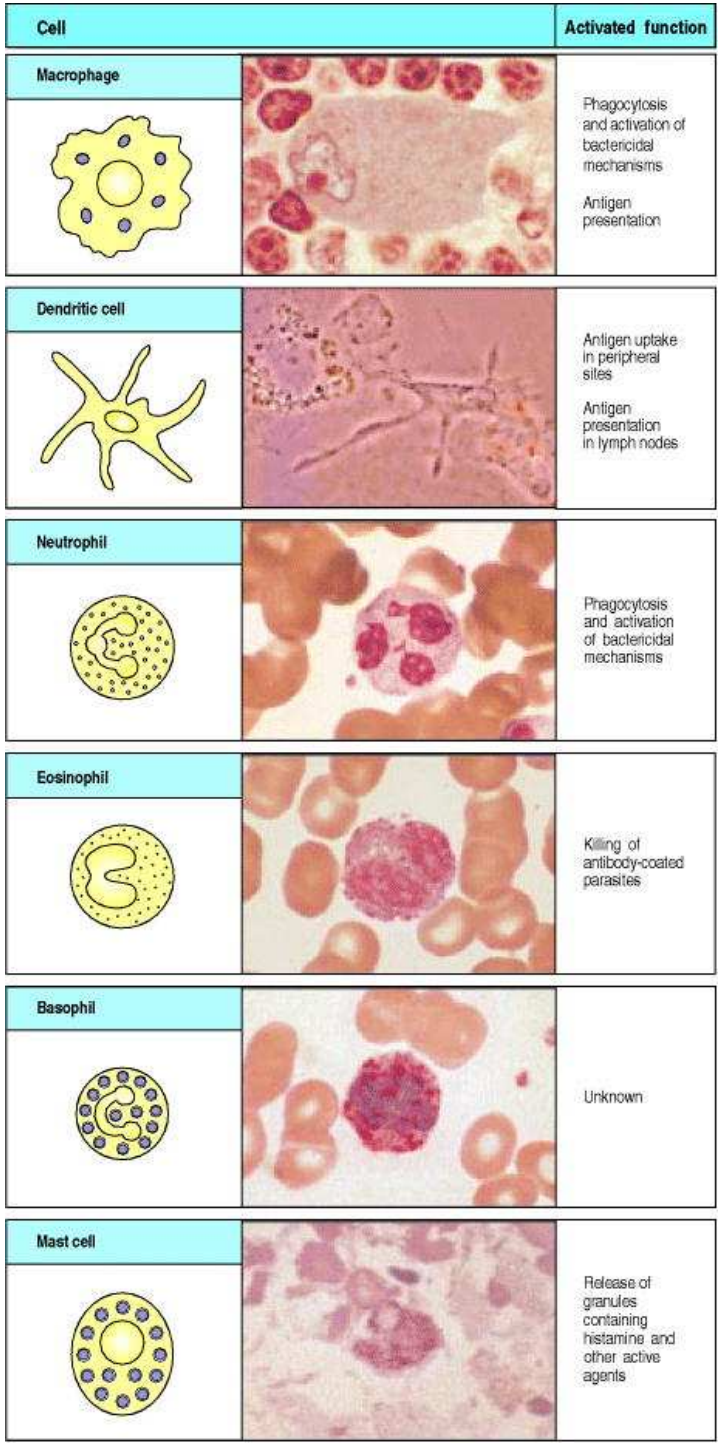


Figure 1-3 Immunobiology, 7ed. (© Garland Science 2008)

All cellular elements of the blood arise from HSC in bone marrow.

Myeloid cells in innate and adaptive immunity.



The distribution of lymphoid tissues in the body.

Lymphocytes arise from stem cells in bone marrow.

They migrate from these tissues and are carried in the bloodstream to the peripheral or secondary lymphoid organs.

The peripheral lymphoid organs are the sites of lymphocyte activation by antigen, and lymphocytes recirculate between the blood and these organs until they encounter antigen.

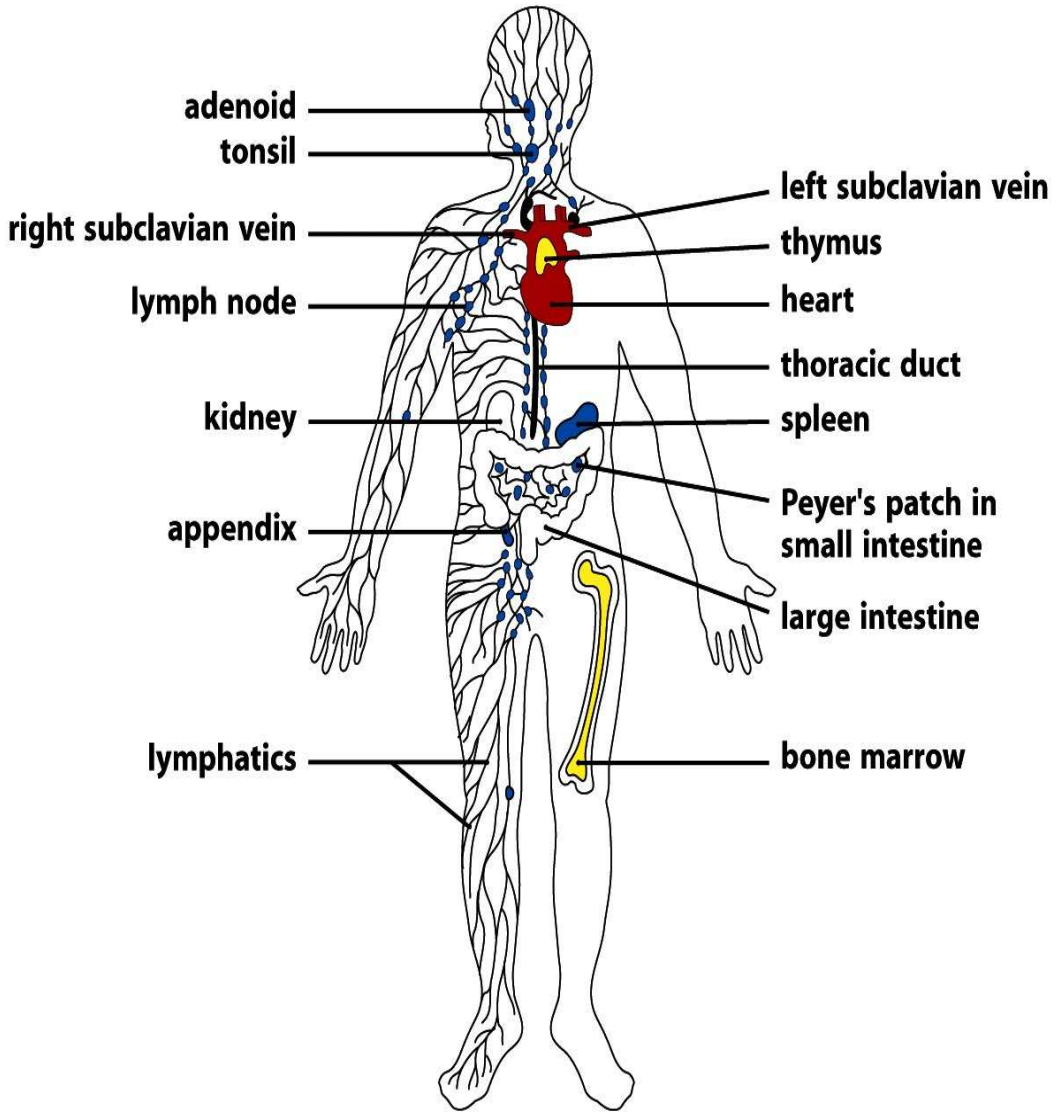


Figure 1-7 Immunobiology, 7ed. (© Garland Science 2008)

Circulating lymphocytes encounter antigen in peripheral lymphoid organs.
Naive lymphocytes encounter their specific antigen, draining from an infected site in the foot. These are called draining lymph nodes, and are the site at which lymphocytes may become activated by encountering their specific ligand.

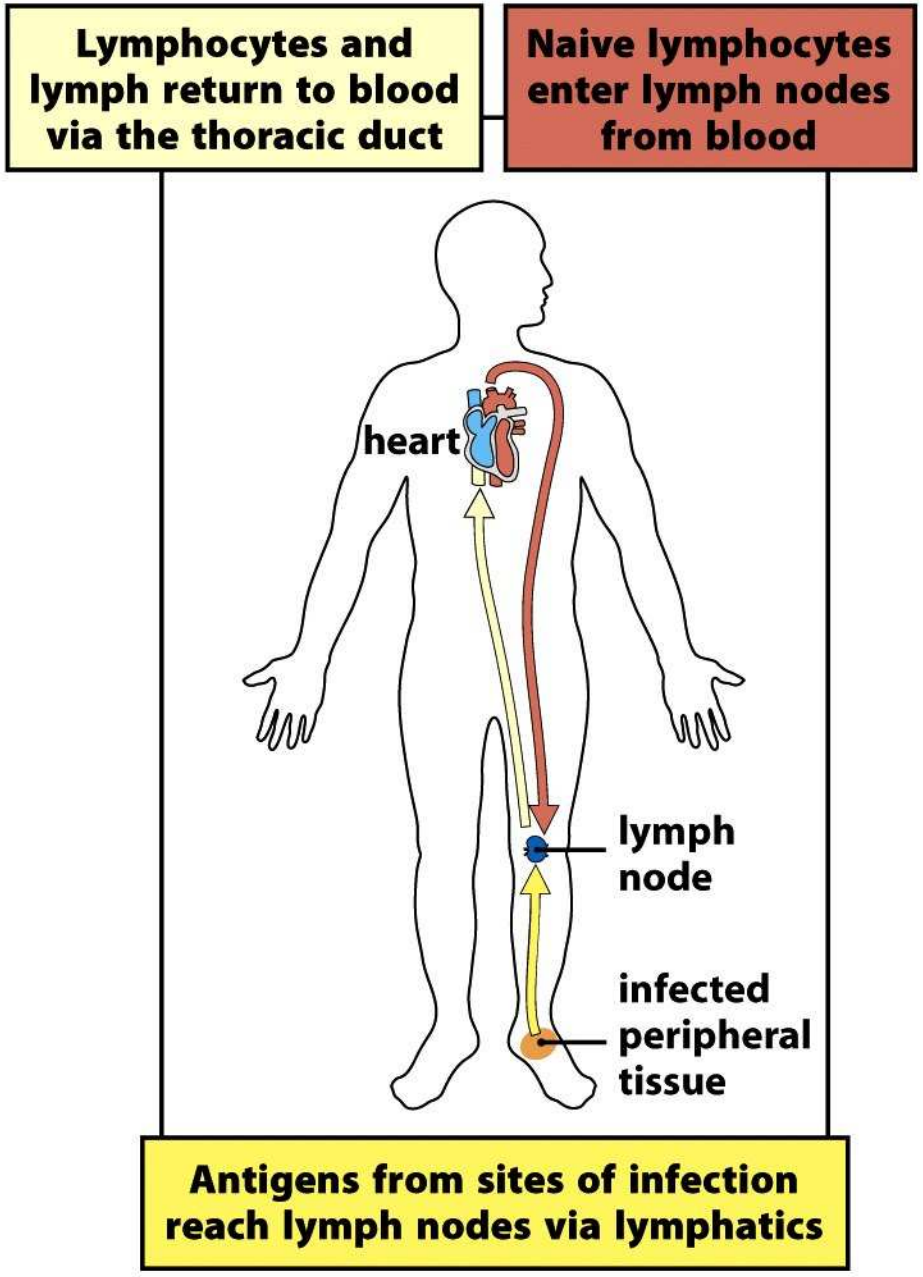


Figure 1-17 Immunobiology, 7ed. (© Garland Science 2008)

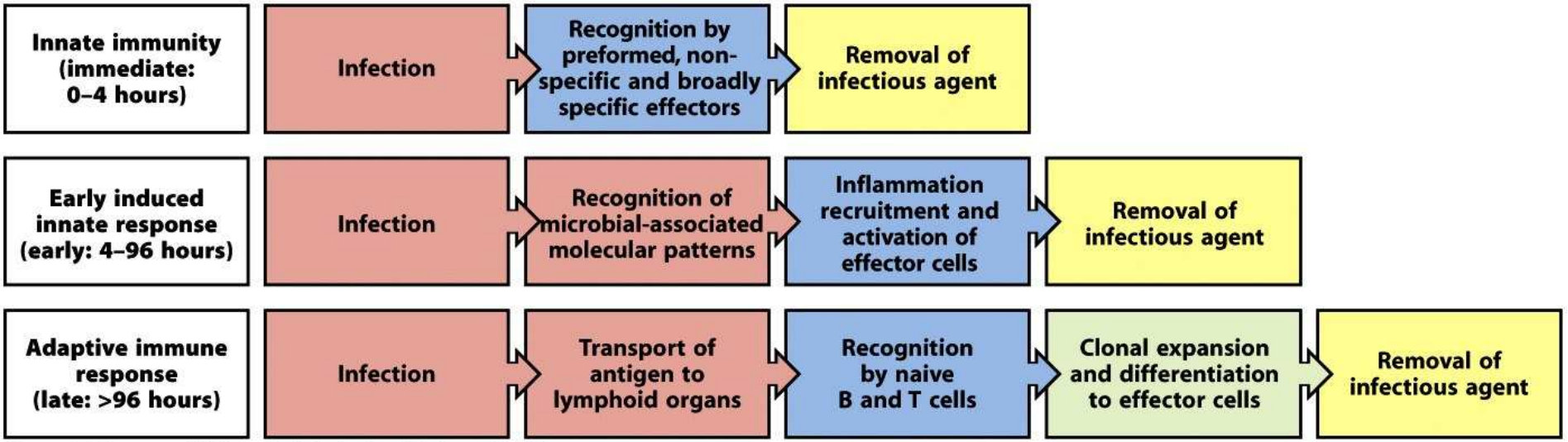


Figure 2-1 Immunobiology, 7ed. (© Garland Science 2008)

The response to an initial infection occurs in three phases.

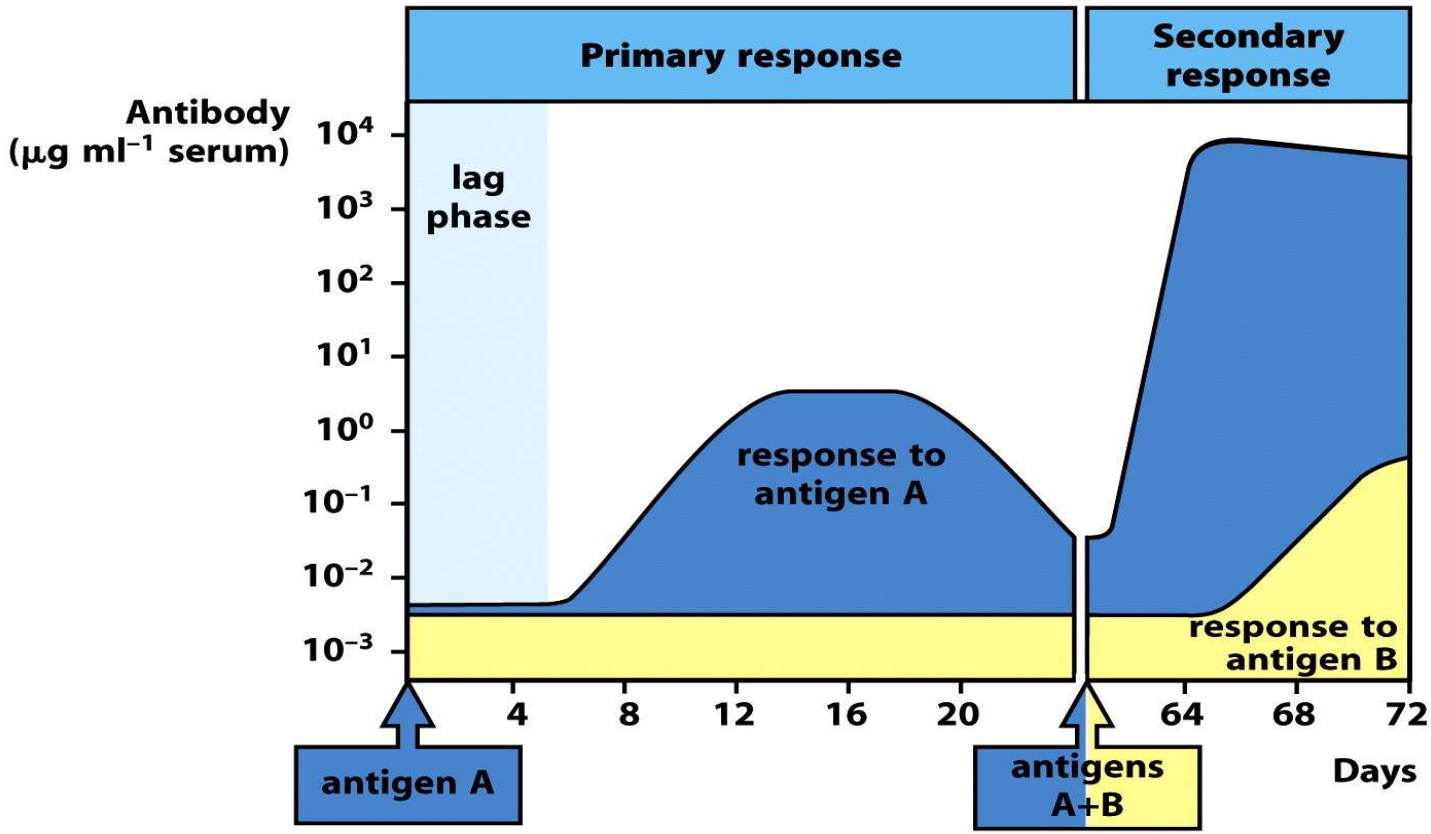


Figure 1-24 Immunobiology, 7ed. (© Garland Science 2008)

The course of a typical immune response.

First encounter with an antigen produces a primary response.

This illustrates **immunological memory**, the ability of the immune system to make a second response to the same antigen more efficiently and effectively, providing the host with a specific defense against infection. This is the main reason for giving booster injections after an initial vaccination.

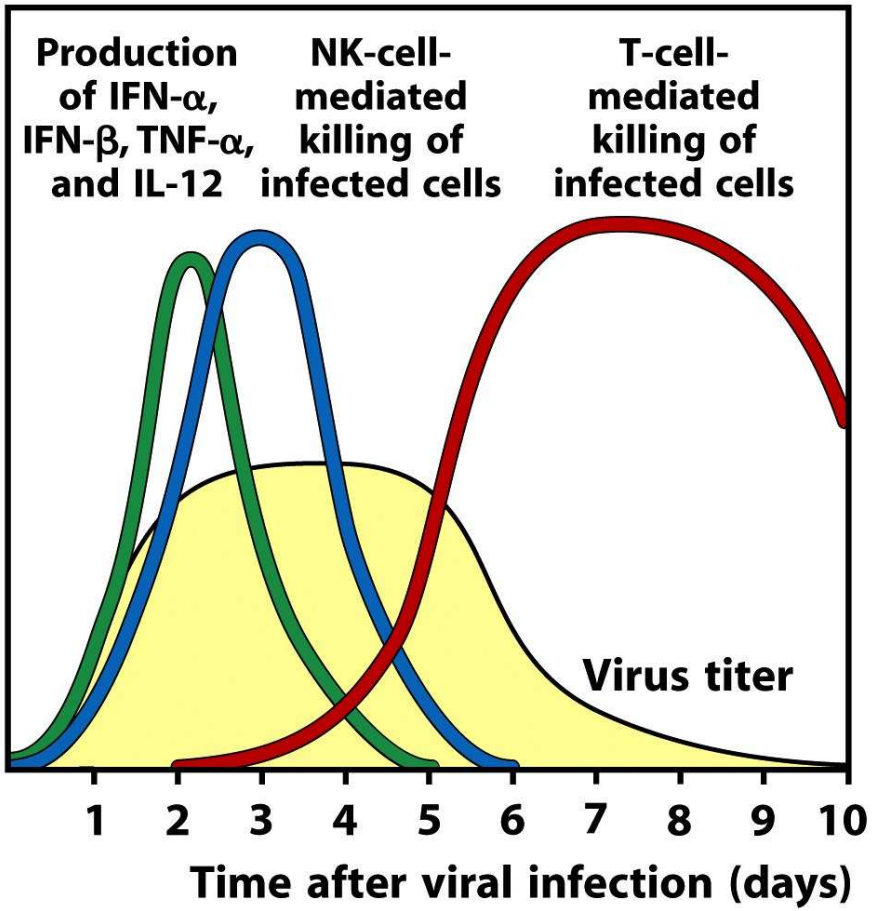


Figure 2-55 Immunobiology, 7ed. (© Garland Science 2008)

Natural killer cells (NK cells) are an early component of the host response to virus infection. Experiments in mice have shown that IFN-α, IFN-β, and the cytokines TNF-α and IL-12 appear first, followed by a wave of NK cells, which together control virus replication but do not eliminate the virus. Virus elimination is accomplished when virus-specific CD8 T cells are produced. Without NK cells, the levels of some viruses are much higher in the early days of the infection, and can be lethal unless treated vigorously with antiviral compounds.

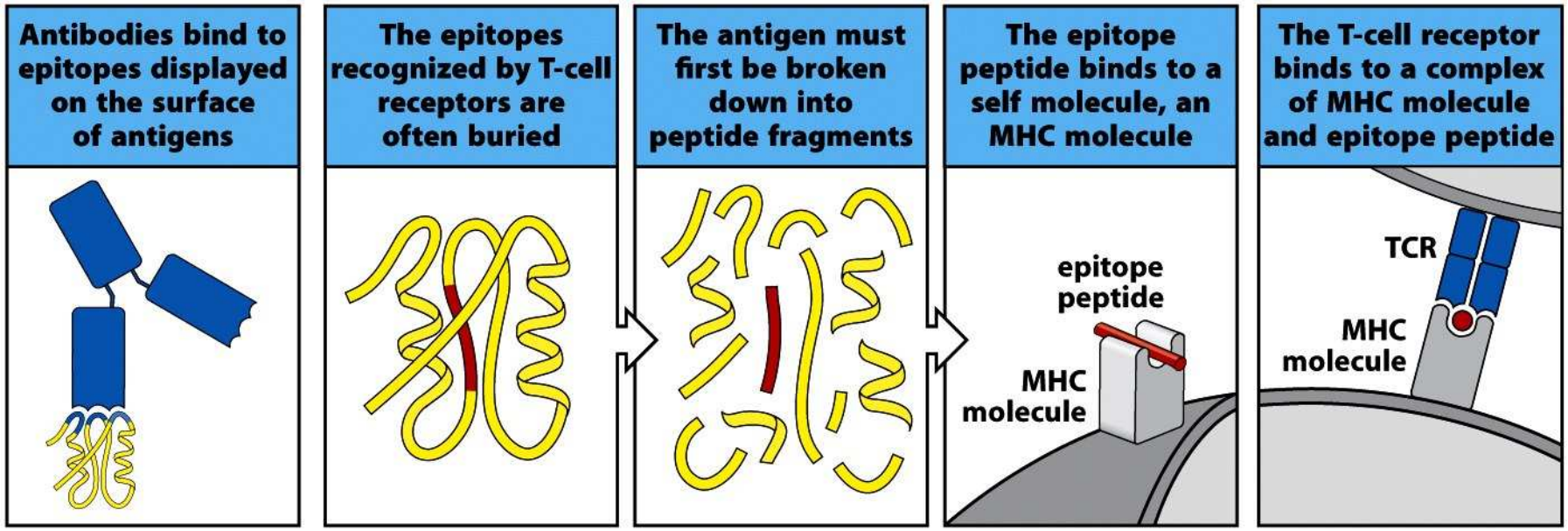


Figure 1-16 Immunobiology, 7ed. (© Garland Science 2008)

Immune Surveillance

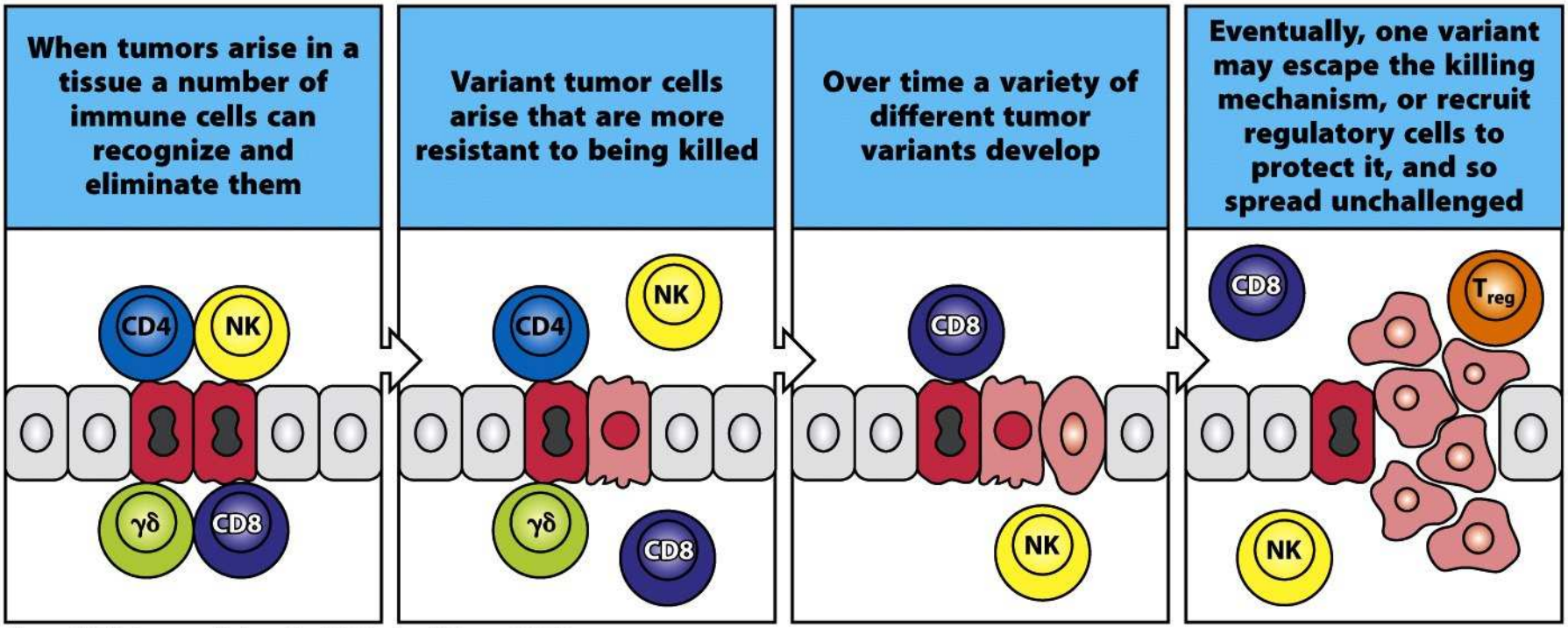


Figure 15-13 Immunobiology, 7ed. (© Garland Science 2008)

Measuring Immunity:

Did the intervention “hit the target”?
Did the desired immune modulation occur?
Was anti-tumor immunity induced?
Was immune suppression reversed?
Were the target cells/molecules activated?

Did the target cells/molecules get to the tumor site and show activity?

Correlations between Immune Monitoring and Clinical Outcome (*predictive* vs. prognostic)

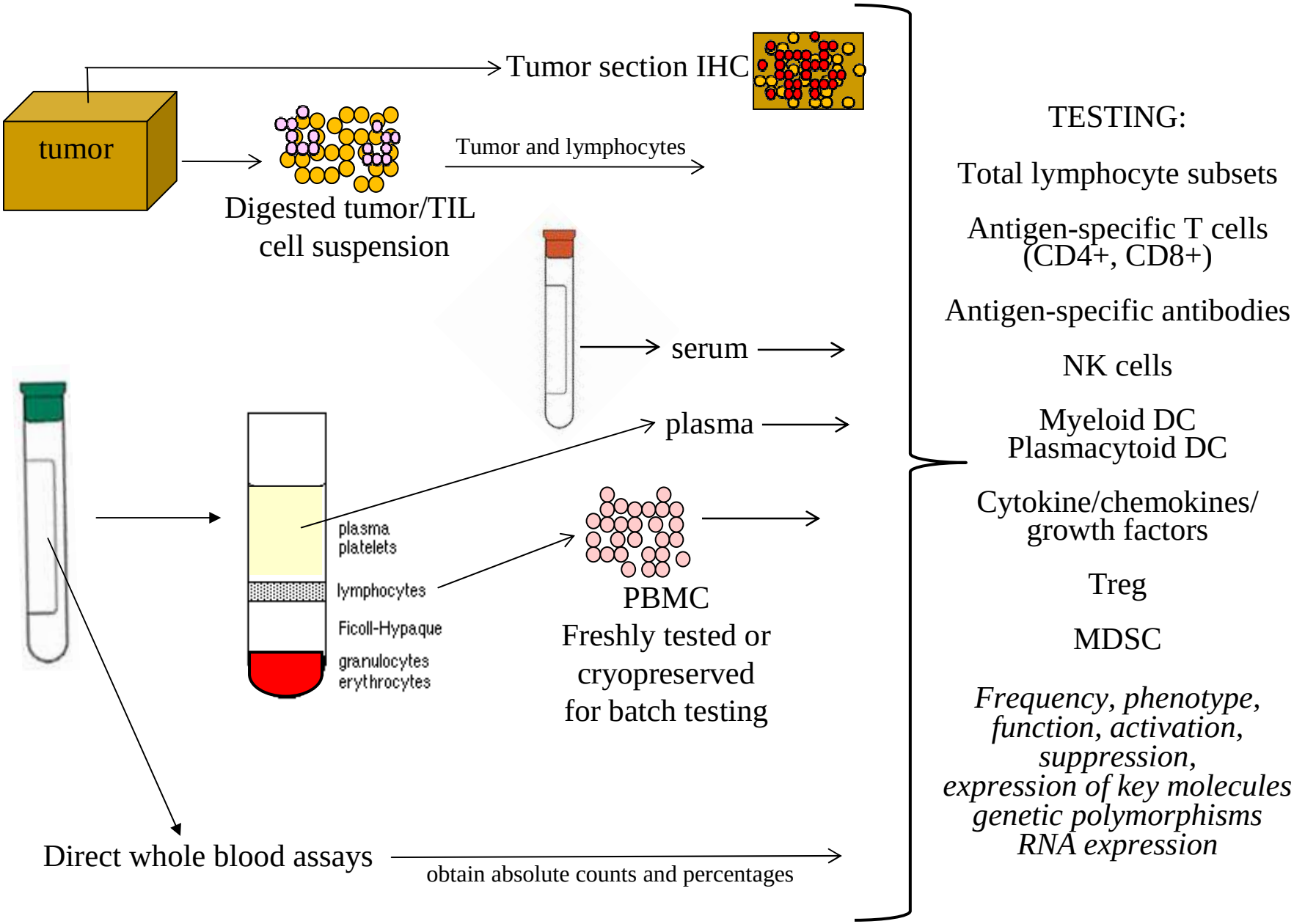
Vaccination of vulvar intraepithelial neoplasia patients with long peptides from HPV16 {Welters, 2010}. Investigators tested lymphocyte proliferation and cytokine production to immunizing antigens as well as circulating regulatory T cells (Treg). Their study showed that the patients with the **strongest proliferation, positive IFNγ and IL-5 production, and lowest Treg were those with complete responses (CR) to therapy.**

A trial testing WT-1 antigen in AML patients indentified significant changes in **WT-1 specific $CD8^+$ T cell frequencies, and more dramatic activation of circulating NK cells in patients with CRs** {Van Tendeloo, 2010}.

A recent study testing a multi-peptide vaccine in renal cancer patients demonstrated that **inhibition of Treg** strengthened antitumor immunity, ***frequencies of specific subsets of MDSC at baseline were critical*,** and that improved clinical outcome correlated with **a larger number of peptides** responded to {Walter, 2012 }. This last observation has been made in previous multi-peptide vaccine trials {Banchereau, 2001}.

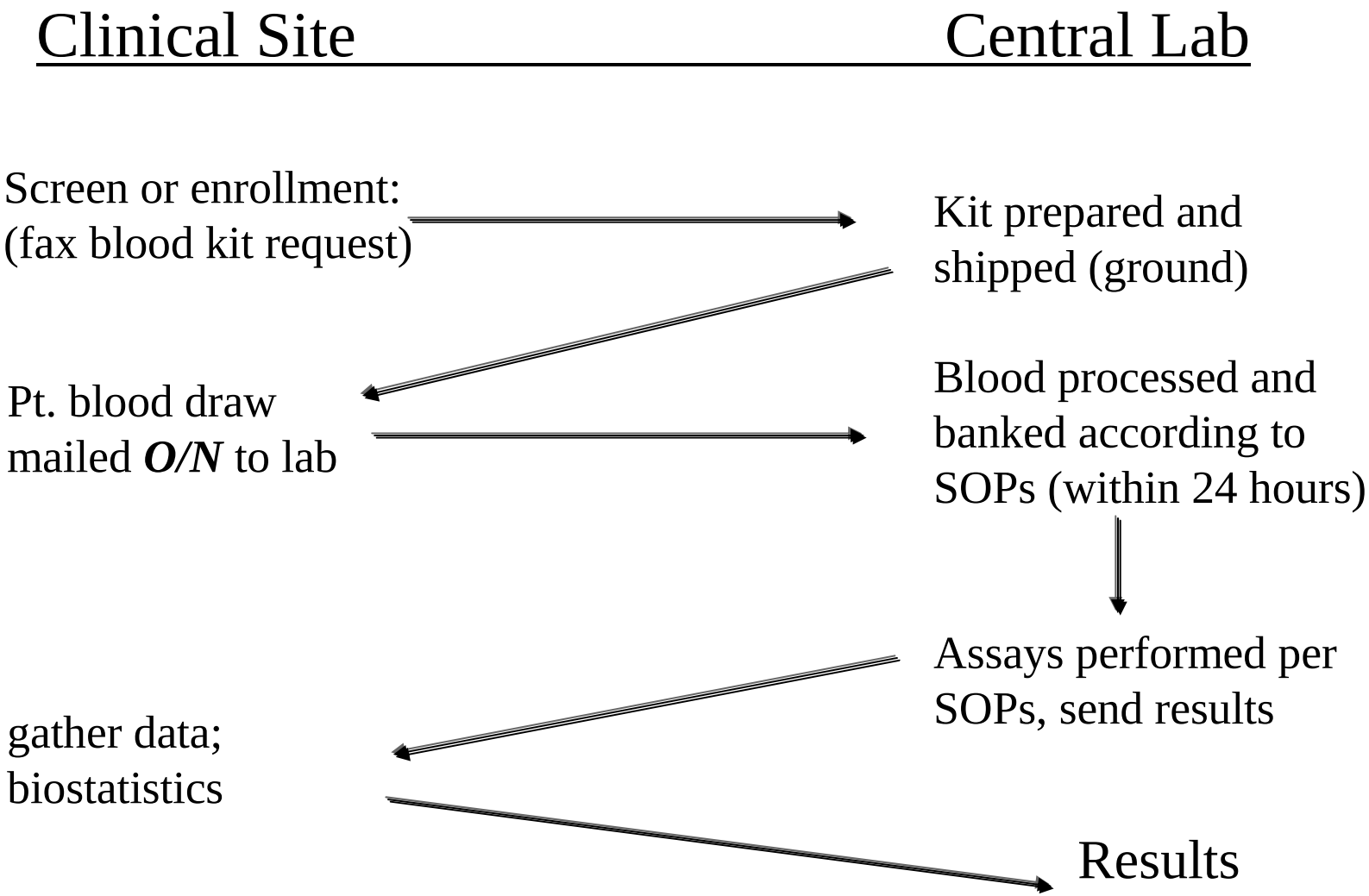
In patients treated with ipilimumab, a number of groups have examined humoral and cellular responses from peripheral blood. Recently, **integrated humoral and $CD8^+$ T cell response to the shared, immunogenic cancer/testes antigen NY-ESO-1** was shown to be a significant immunologic measure correlating with clinical outcomes {Yuan, 2011}. In a related publication, circulating T cells specific to **NY-ESO-1 and MART-1** antigens (but not MAGE-A3 or survivin antigens) were correlated with melanoma patient survival {Weide, 2012}.

A final example of an immune measure that several independent groups have correlated to clinical outcome is "epitope spreading" or "**determinant spreading**". This acquisition of T cell and antibody reactivity to shared antigens or epitopes other than those used in a vaccine, has been observed in melanoma {Ranieri, 2000; Butterfield, 2003; Ribas, 2004; Butterfield, 2008}, renal cancer {Wierecky, 2006 } and breast cancer {Disis, 2002 }.



Patient-derived specimens used in immunologic monitoring

Central Immunology Laboratory



Healthy Donor Absolute Counts and Percentages

CD3	CD3	CD3/CD4	CD3/CD4	CD3/CD8	CD3/CD8	CD3	CD3	CD16+CD56	CD16+CD56	CD19	CD19
% positive	cells/µL	% positive	cells/µL	% positive	cells/µL	% positive	cells/µL	% positive	cells/µL	% positive	cells/µL
90	1918	63	1345	26	562	90	2088	6	104	4	100
81	1077	49	644	29	388	82	1081	6	76	12	163
71	1505	62	1311	9	192	70	1542	9	206	20	446
86	2324	59	1576	27	729	85	2512	5	143	10	288
82	1442	50	887	27	478	82	1428	9	151	9	159
79	1301	50	824	31	511	80	1291	9	143	11	181
77	1307	45	772	31	527	77	1309	7	114	16	269
81	986	58	706	23	279	81	992	6	68	14	165
90	2178	67	1618	23	548	90	2189	3	76	7	171
78	2027	45	1175	29	766	77	1886	5	114	18	438
76	1155	63	965	13	193	78	1183	8	127	14	214
77	1372	48	842	30	527	76	1400	10	180	14	251
75	1017	56	763	16	211	76	1065	9	120	15	216
83	1916	62	1420	20	458	83	1929	4	98	13	301
67	1498	47	1049	21	457	67	1520	16	373	16	372
83	2143	62	1609	19	501	82	2257	7	198	11	301
82	1300	39	624	43	687	82	1407	11	183	8	129
76	1222	54	865	21	337	77	1290	11	185	12	200
73	1097	54	808	17	264	74	1174	16	248	11	168
86	1784	63	1321	21	432	85	1814	4	90	11	236

CD3+:	986-2,512 cells/ul	(2.5x)
CD3+/CD4+:	39-67%	(1.7x)
CD3+/CD8+:	9-43%	(4.7x)
CD19:	4-20%	(5.0x)
CD16+/CD56+:	3-16%	(5.3x)

ImmunoAssay Proficiency Panel Program

About the ImmunoAssay Proficiency Panel Program

The program's objectives are:
1)to offer an external validation program, and
2)to enhance assay harmonization.

The Proficiency Panel Program seeks to identify:
issues and deficiencies of current assay practices;
sources for assay variability within and among institutions;
protocol details that optimize assay performance.

Cytokine ELISPOT assay
Intracellular Cytokine Staining
Multimer (Tetramer/Pentamer) Staining
Luminex Multiplex cytokine analysis
Myeloid-derived suppressor cells (MDSC)

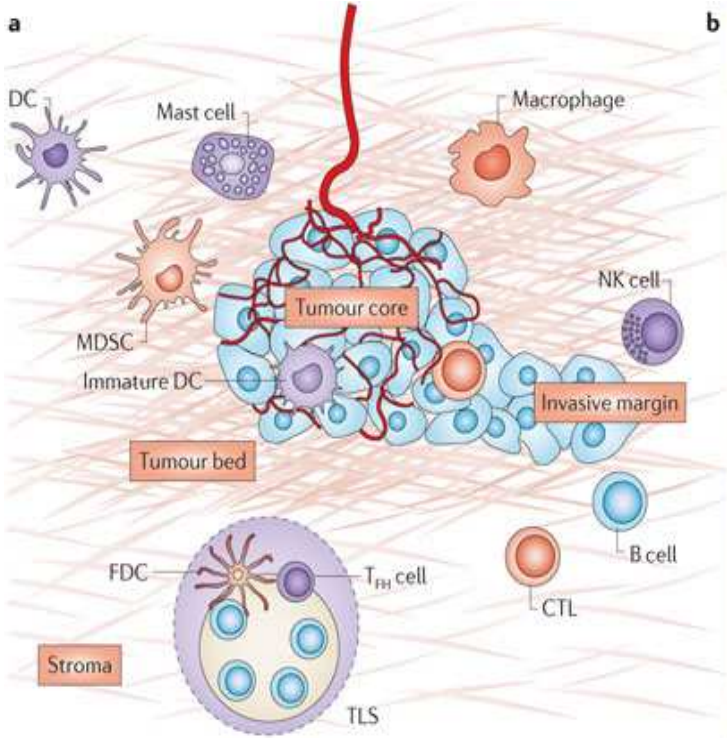
Central laboratory services, cells, and shipping are provided by the Immunology Quality Assurance Center (IQAC) of the Duke Human Vaccine Institute (Durham, NC) under the leadership of Dr. Thomas Denny.

Approaches to addressing inherent variability in immunologic monitoring of clinical trials

Source of Variability	Recommendation
Patient	Save DNA/RNA/cells/tumor to understand host variation include healthy donor control
Blood draw	Standardized tubes and procedures
Processing/cryopreservation/ thaw	Standardized procedures and reagents
Cellular product	Phenotypic and functional assays to characterize the individual product, development of potency assays
Assay choice	Standardized functional tests
Assay conduct	Standardized operating procedures (SOPs)
Assay analysis	Appropriate biostatistical methods
Data reporting	Full details, controls, quality control/assurance (QA/QC) MIATA guidelines
Newest, non-standardized technology	Sufficient blood/tissue to interrogate the samples *now*, as well as *later*, to generate new hypotheses

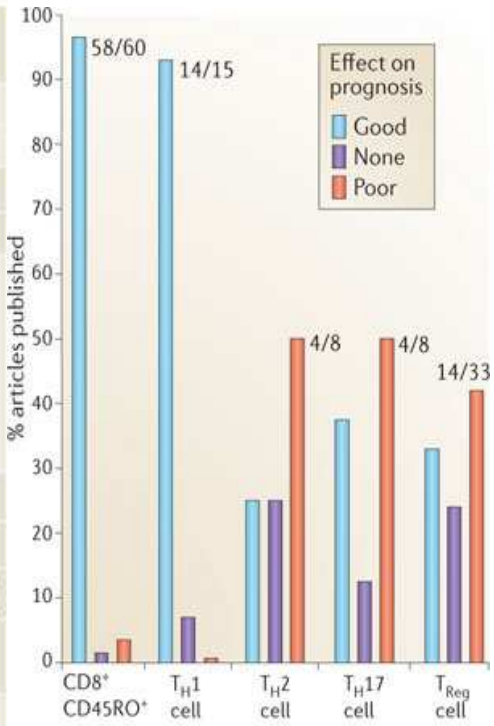
Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers, CCR 2011

Immunoscore



b

Immune contexture	Parameters: positive association with survival
Type	CTLs ($CD3^+CD8^+$)
	Memory T cells ($CD45RO^+$)
Location	Core of the tumour
	Invasive margin
Density	Number of cells per mm^2: 1, 10, 100, 1,000, 10,000 — $CD3^+_{CT}$, $CD3^+_{IM}$, $CD8^+_{CT}$, $CD8^+_{IM}$, $CD45RO^+_{CT}$, $CD45RO^+_{IM}$
Functional orientation	T_H1 cell-associated factors (IFNγ, IL-12, T-bet and IRF1)
	Cytotoxic factors (granzymes, perforin and granulysin)
	Chemokines (CX3CL1, CXCL9, CXCL10, CCL5 and CCL2)
	T_H17 cells, T_{Reg} cells and T_H2 cells have a variable effect on survival, depending on tumour type
TLS	Presence and quality



Nature Reviews | Cancer

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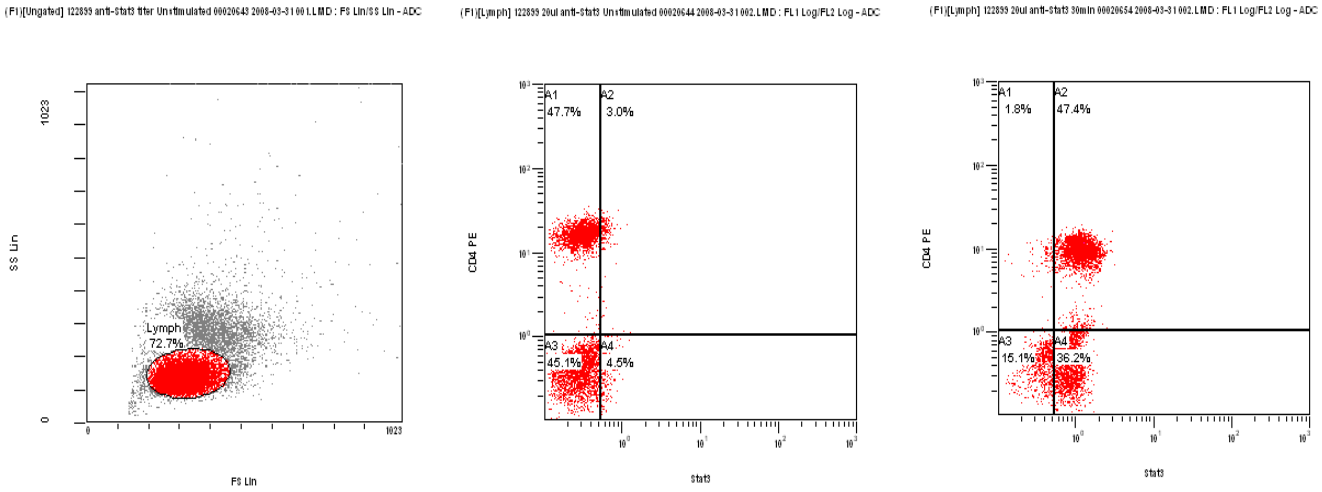
Parameters of the tumor “immune contexture” or “Immunoscore” that predict a good prognosis. Enumeration/localization of the CD3+/CD8+/CD45RO memory CD8+ T cells infiltrating the tumor.

J. Galon, W. Fridman

STAT-3 Phospho-flow assay:



FSC vs. SSC Lymphocytes	Un-stimulated CD4/Stat3	30 minutes stimulation

Did the signal transduction inhibitor “hit the target”?

Luminex multiplex Cytokine Analysis

This 30-plex kit for the simultaneous measurement of:

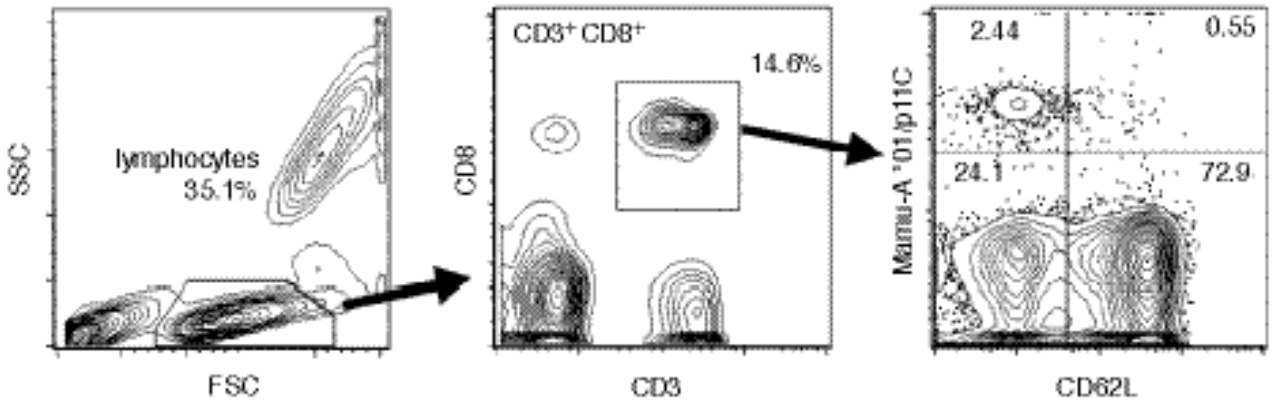
IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40⁄p70), IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, RANTES, MCP-1, VEGF, G-CSF, EGF, FGF-basic, and HGF

in serum, plasma, or tissue culture supernatant.

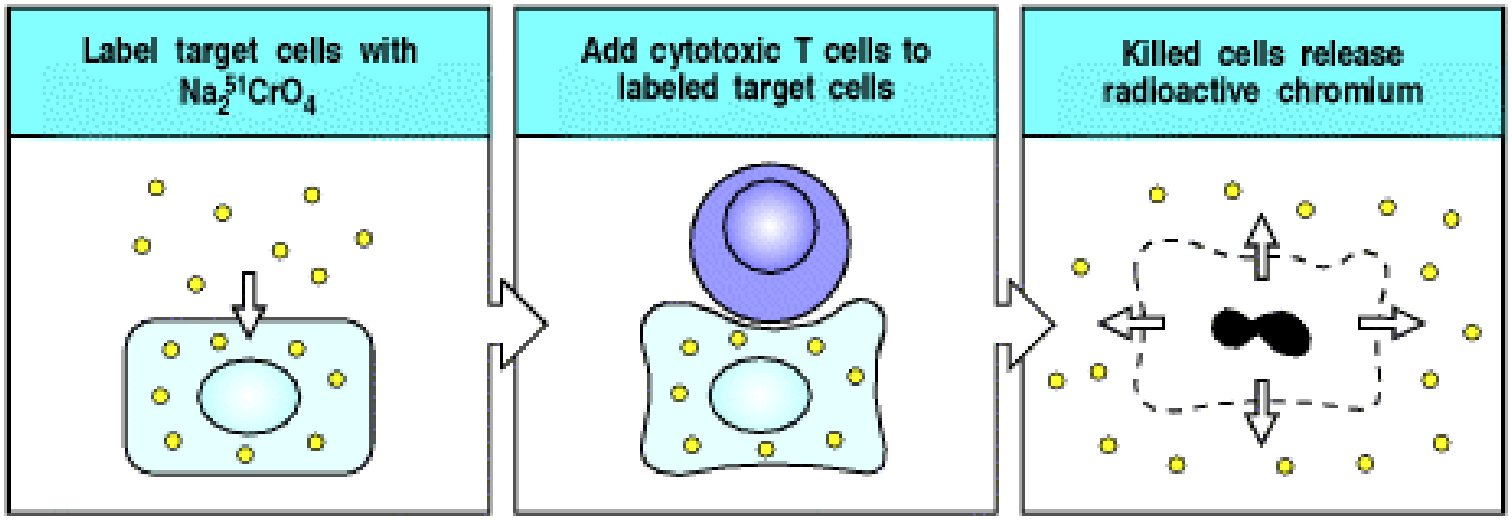
Sample size: 50 ul

(Local vs. systemic measures)

MHC-Peptide Tetramers to Visualize Antigen-Specific T Cells



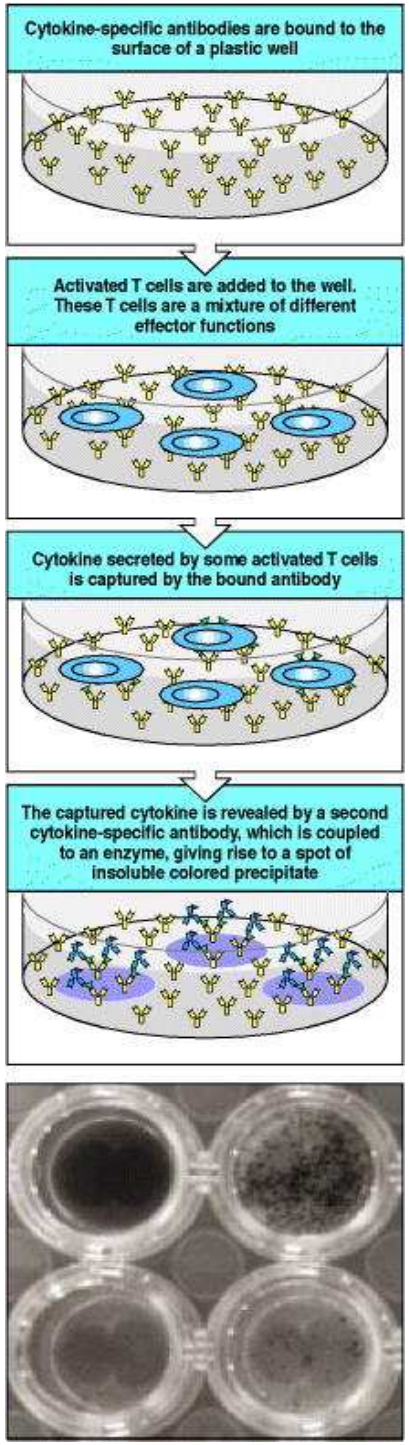
Enumerates the antigen-specific T cells.
Not a functional assay—are they cytotoxic?
Cytokine producers (Th1/Th2)?



Cytotoxic T-cell activity is often assessed by chromium release from labeled target cells. Target cells are labeled with radioactive chromium as $Na_2{}^{51}CrO_4$ and exposed to cytotoxic T cells. Cell destruction is measured by the release of radioactive chromium into the medium, detectable within 4-6 hours of mixing target cells with T cells.

The frequency of cytokine-secreting T cells can be determined by the ELISPOT assay.

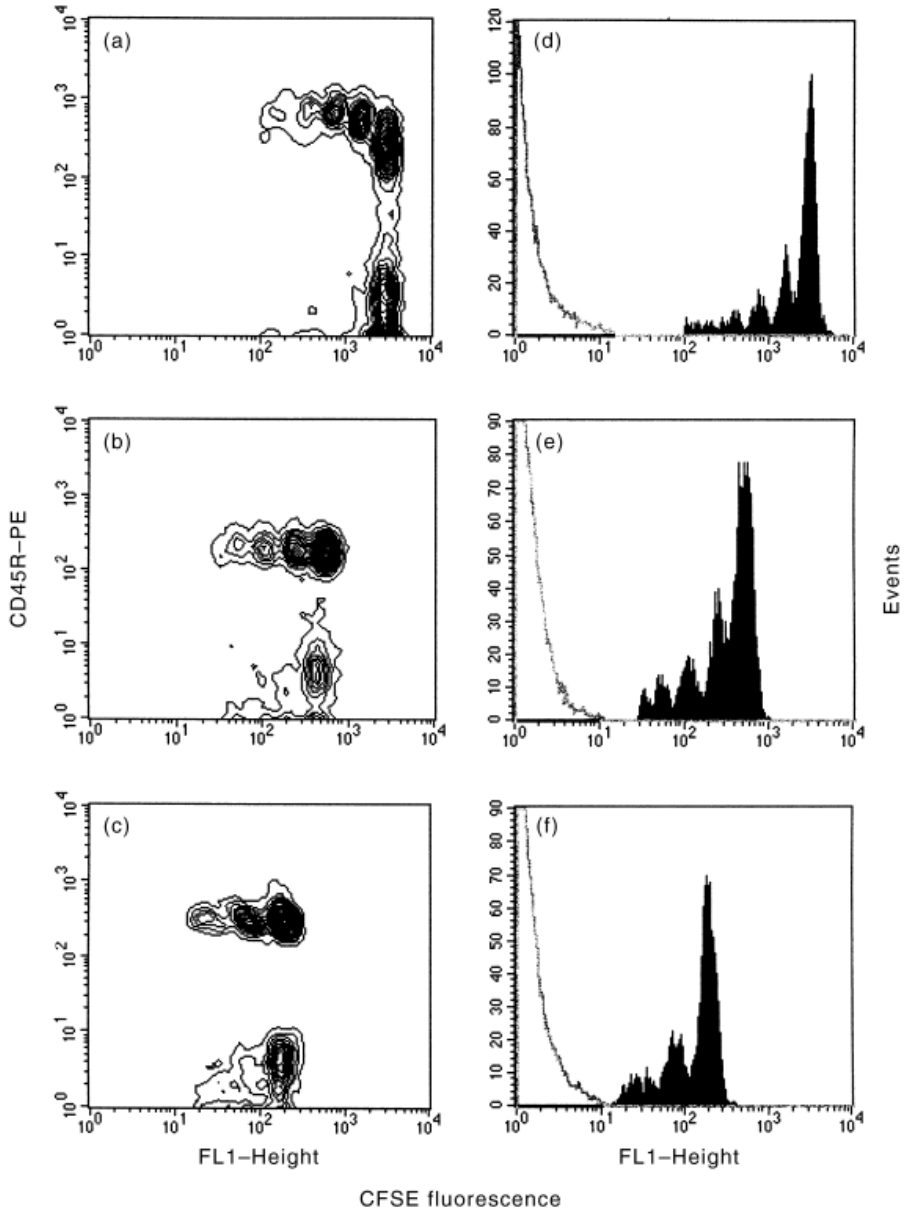
Each T cell that originally secreted cytokine gives rise to a single spot.



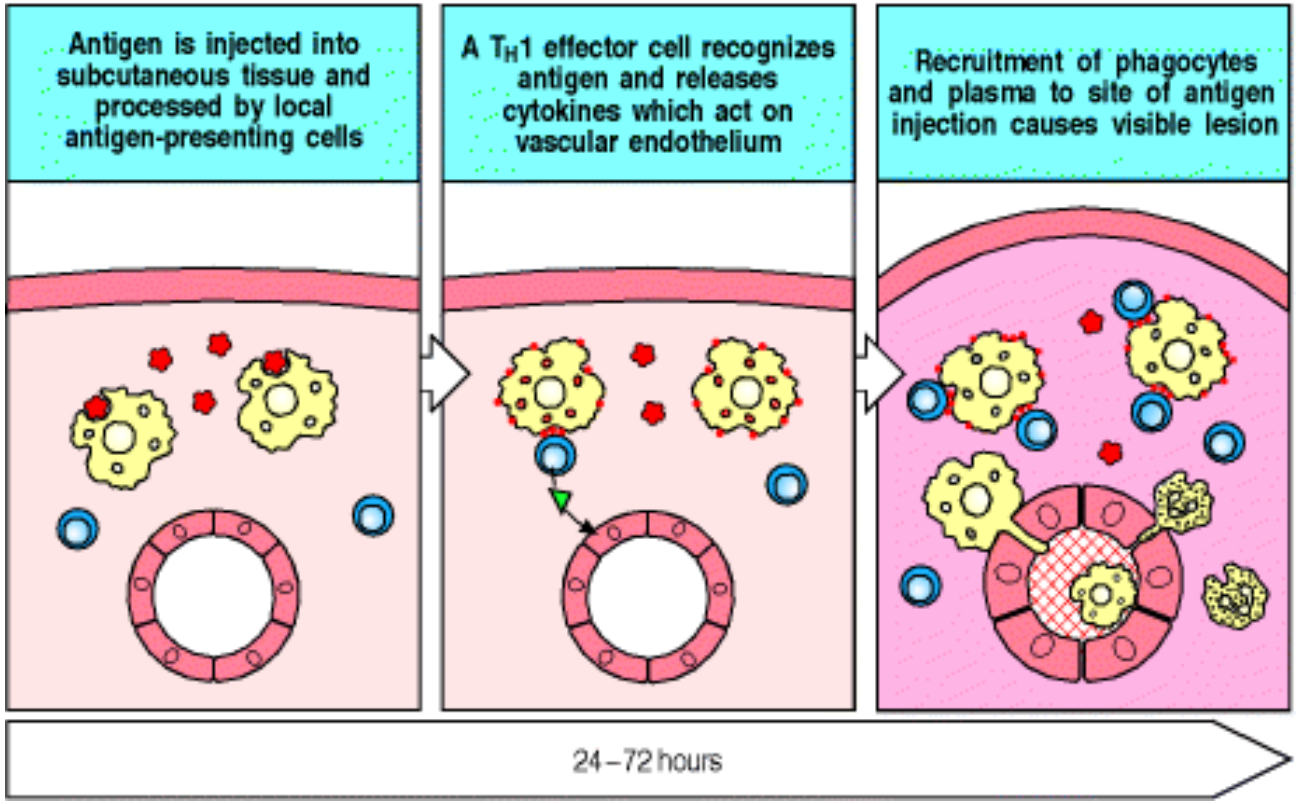
CFSE Proliferation

This is a cell division analysis procedure based on the quantitative serial halving of the fluorescent dye CFSE.

The technique can be used both *in vitro* and *in vivo*, allowing eight to 10 successive divisions to be resolved by flow cytometry.



Delayed Type Hypersensitivity Reaction



The stages of a delayed-type hypersensitivity reaction. involves uptake, processing, and presentation of the antigen by local antigen-presenting cells.
Because these specific cells are rare, it can take several hours for a T cell of the correct specificity to arrive.
These cells release mediators that activate local endothelial cells, recruiting an inflammatory cell infiltrate dominated by macrophages and causing the accumulation of fluid and protein.

Analyses of delayed-type hypersensitivity (DTH) biopsies *in situ*.

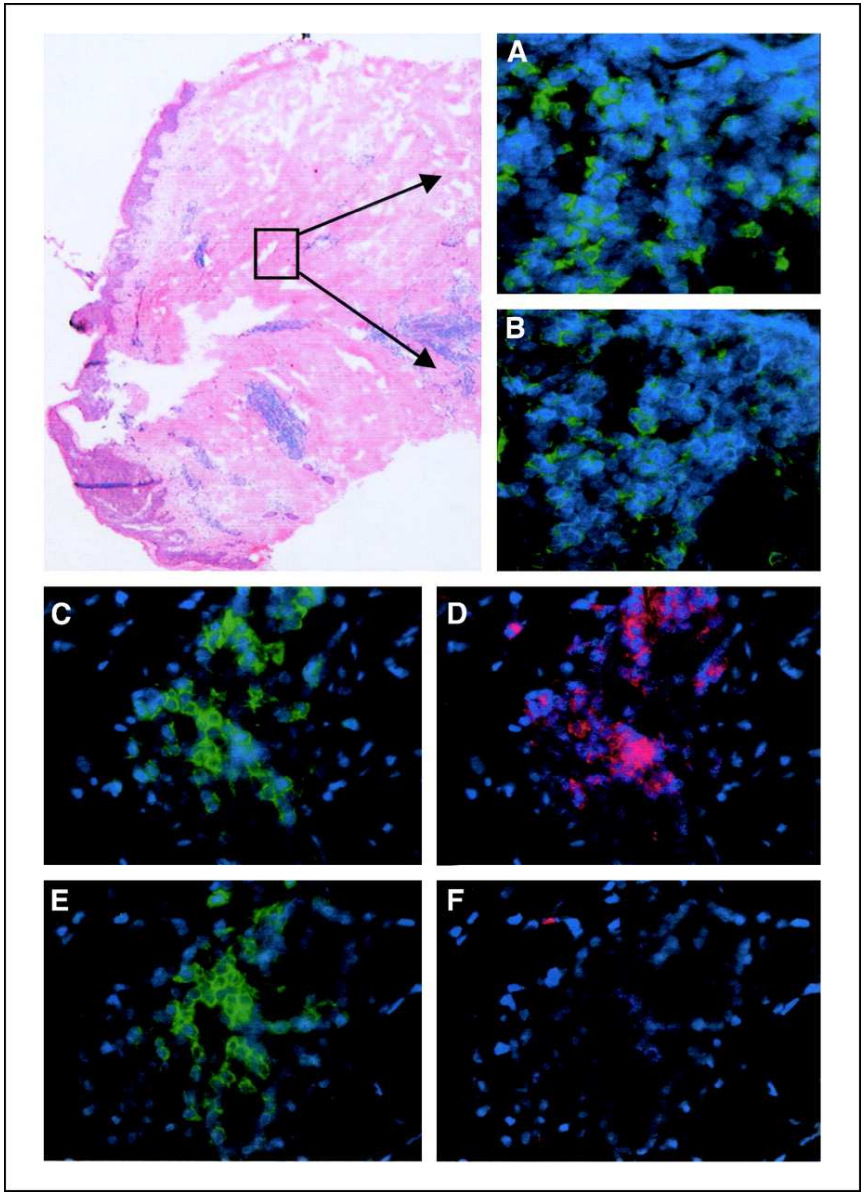


Fig 2. Analyses of delayed-type hypersensitivity (DTH) biopsies in situ. Staining of the DTH sections of a vaccinated patient revealed infiltrating clusters of (A) CD8 and (B) CD4 T cells. In situ tetramer staining on cryosections of biopsies: sections were stained with CD8 in green (C and E) in combination with tetramer tyrosinase (D) or gp100:280 (F) in red.

de Vries I J M et al. JCO 2005;23:5779-5787

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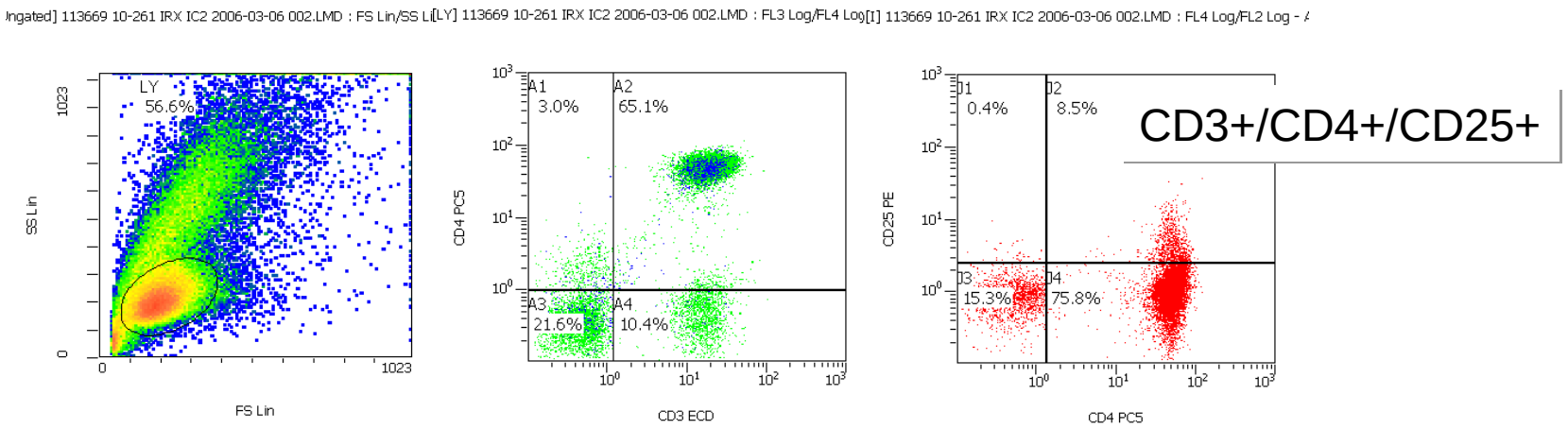
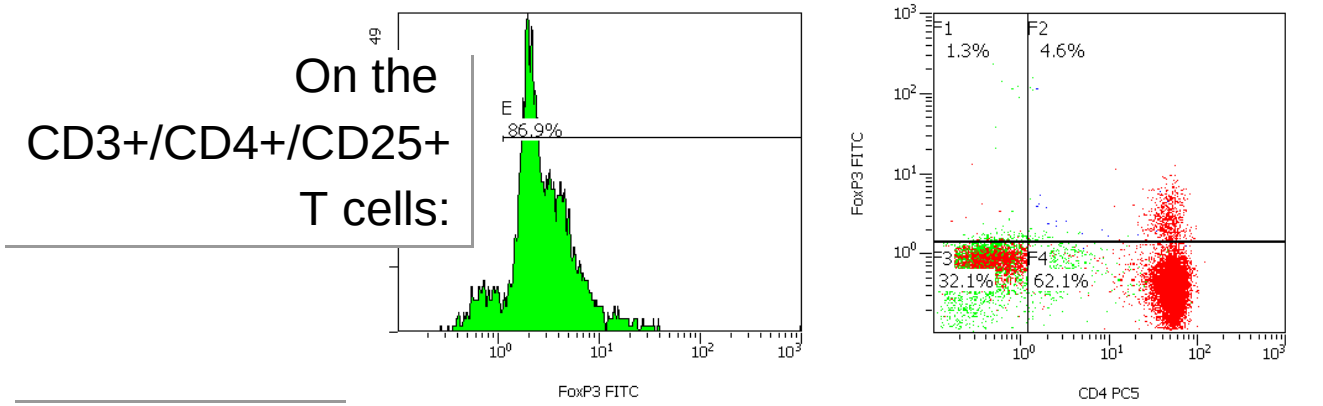
Regulatory T cells (Treg)

flow cytometry phenotype



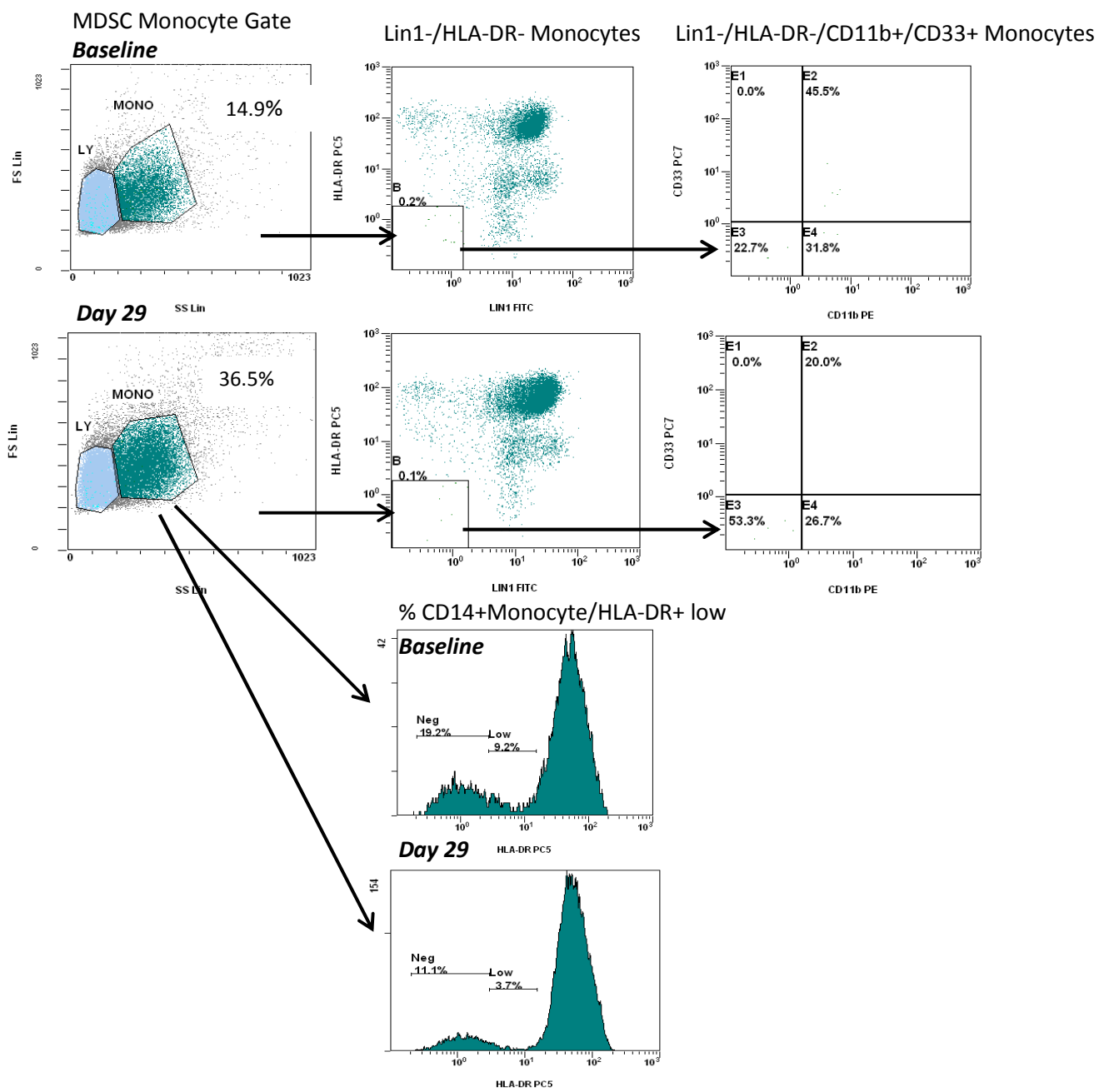
CD3+/CD4+/CD25+

On the CD3+/CD4+/CD25+ T cells:



Total FoxP3 by CD4+: histogram and dot plot

Myeloid-Derived Suppressor Cells (MDSC)



Measuring Immunity in Immunotherapy Clinical Trials:

- Was the cytokine induced (right time/place/level)?
- Did the vaccine activate tumor-specific T cells?
- Did the adoptively transferred effector cells survive/traffic to the tumor/kill the tumor?
- Was immune suppression reversed?
- Were the target cells/molecules activated?
- Did the target cells/molecules get to the tumor site and show activity?

- *Was the therapeutic intervention an improvement?*
- *Why or why not?*