



## Editorial

By Dr John Wijdenes

Most of this Newsletter is dedicated to the Th1/Th2/Th3/Tr1 cells, a field of research in which many cytokines play an important role and I thank Dr. Yssel and Dr. Vermot-Desroches for their help in the preparation of this overview. Diacclone can provide many of the necessary tools to investigate the role of cytokines in T-cell differentiation in the form of ELISA's, Elispot kits and cytoplasmatic staining. Our research is still ongoing with the hope to provide you specific markers for flow cytometry. On the last page you can read about our updated website with search engine and technical sheets for all the Diacclone products. Please contact us if you need more information.

### Th1/Th2 immunological concepts

#### Historical background

Since their original description by Mosmann and Coffman (1) in the mid eighties, the concept of CD4 T helper type 1 (Th1) and Th2 lymphocyte subpopulations has dominated the field of immunology and their discovery has provided a basis to explain the reciprocal nature of polarized immune responses. The different roles that Th1 and Th2 lymphocytes play in normal and pathological immune responses are in large part due to the production of cytokines. These are soluble and low molecular weight proteins with pleiotropic and redundant functions.

#### Cytokines associated with Th1 or Th2 responses

When a naïve precursor cell (Thp) recognises an antigen presented by an antigen-presenting cell (APC), it enters into a phase of both production and consumption of IL-2 via an IL-2 autocrine pathway. At this stage, the T cell has the capacity to polarize towards a Th1 or Th2 phenotype. During this differentiation process, the genes for several effector cytokines such as IL-4, IFN- and IL-10 are

expressed. Once fully differentiated, activated Th1 cells produce IL-2, IFN- and lymphotoxin (LT, TNF- ), whereas activated Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13 (2). The skewing of an immune response to either a Th1 or Th2 cell-mediated response is dependent on the interaction of cytokines, chemokines and adhesion molecules with their respective receptors involving T cells as well as APC. Under certain conditions, these stimuli may also lead to a clonal T cell population (Th0) producing both type-1 and type-2 cytokines.

Type-1	Type-2
IL-2	IL-2
IL-3	IL-3
IL-12	IL-4
IL-15	IL-5
IL-18	IL-6
IFN $\gamma$	IL-10
TNF $\alpha$	IL-13
TNF $\beta$	IL-18
	TNF $\alpha$

Table 1

Cytokines play a major role in the differentiation of T helper Type-1 or Type-2 cells (table 1) (3). IL-12 is a cytokine which is essential to Th1 differentiation (4). It is produced by monocytes and dendritic cells and it is a strong inducer of IFN- production by T cells and natural killer cells. A number of other cytokines, produced by APC, play a role in the process of Th1 differentiation as well. Because a functional IL-12 receptor consists of two chains, an IL-12R 1 and an IL-12R 2 chain, naïve T

## Contents

- Editorial
- Th1/Th2 immunological concepts
- Visit our new website
- Contacts

cells, which only express the IL-12R 1 chain, do not respond to IL-12. The expression of the IL-12R 2 chain is induced on naïve T cells by IFN- $\gamma$ , which renders these cells responsive to the IFN- $\gamma$ -inducing effects of IL-12 (5). Moreover, IL-12 acts in concert with IL-18 (6) and IL-23 (7) two cytokines with strong IFN- $\gamma$ -inducing effects as well. However, none of the cytokines on its own is able to fully induce the differentiation of naïve T cells into Th1 cells. The role of IL-18 is underscored by the finding that the IL-18R is a member of the Toll family of receptors involved in anti-bacterial and inflammatory Th1 cell-mediated responses. Although the role of IL-23 in Th1 differentiation is not yet clear, it is of interest to note that the IL-23R shares a signal transduction component with the IL-12R. To date, the only known cytokine to drive the differentiation of naïve T cells into Th2 cells is IL-4, although some controversy still exists as to the nature of the IL-4 producing cells in the early immune response (8).

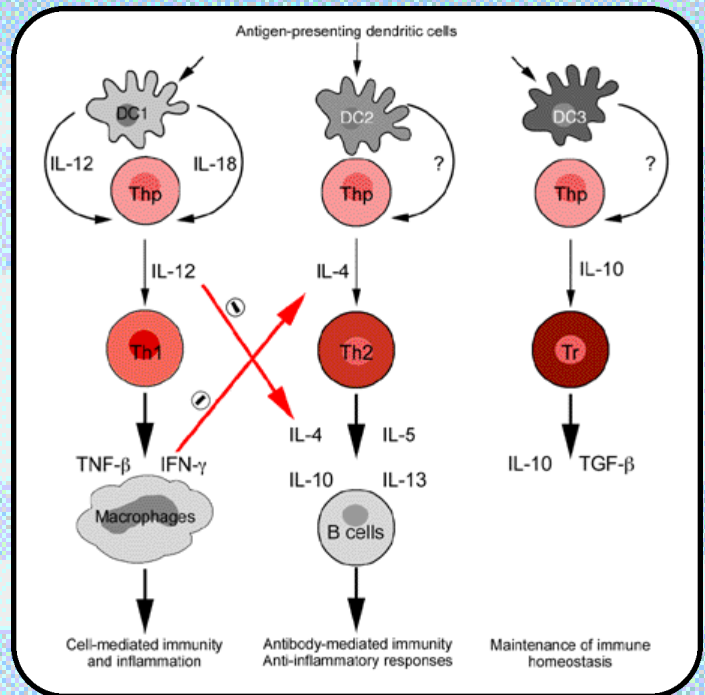
### Th1/Th2 cell activities

Th1 cells induce protective, phagocyte-dependent, immune responses toward intracellular micro-organisms, which involve the induction of cell-mediated delayed type of hypersensitivity reactions, as well as isotype switching by B cells to the production of complement fixing and opsonising antibodies. Th2 cells are important for the elimination of extra cellular parasites. They are also involved in the pathogenesis of allergic diseases, as a result of the production of IL-4 and IL-13 which are switch factors for the production of IgG and IgE antibodies by B cells, as well as by the production of IL-5, a potent growth and differentiation factor for eosinophils (2).

Immune responses, mediated by Th1 cells, generally characterized by inflammatory reactions, lead to tissue damage and destruction which are, under normal physiological conditions, counter-balanced by the anti-inflammatory effects of IL-4, IL-10 and IL-13. This property has led to the notion that Th2 lymphocytes play a role as a "damper" of unwanted inflammatory reactions. Under pathological situations, exacerbated Th1 effector responses are associated with the induction of certain inflammatory diseases, such as Crohn's disease, and autoimmune diseases, like rheumatoid arthritis, multiple sclerosis and insulin-dependent diabetes mellitus suggesting that the pathogenesis of these diseases is the result of a lack of immunoregulatory activity (2, 7).

### T regulatory cells

As Type-1 and Type-2 cytokines cross regulate each others' production, this provides a mechanism for regulation of immune responses. It has become clear over the past years that other subpopulations of CD4+ T cells also play an important role in the homeostasis of the immune response.



In particular, T regulatory type 1 (Tr1) cells, characterized by high production levels of IL-10, but low levels of IL-2 and an absence of IL-4 production play a critical role in the normal regulation of intestinal immune responses (9, 10). Another subpopulation of CD4+ T lymphocytes with strong immunosuppressive activity can be generated from myelin basic protein-specific T cells following the oral administration of this protein to mice. These cells, called Th3 cells, secrete high levels of TGF- $\beta$ , but variable amounts of IL-4 and IL-10 and can protect mice from experimental autoimmune encephalomyelitis (11).

Very recently, much attention has been paid to CD4+ CD25+ T cells, a hyporesponsive (anergic) T lymphocyte population with strong immunosuppressive activities, detected at low frequencies in human peripheral blood (12-15), following earlier reports of the existence of such cells in the mouse (16, 17). Like Tr1 and Th3 cells, the CD4+ CD25+ T cells require antigen-mediated T cell receptor triggering for the induction of suppressor activity and, once activated, suppress T cell activation via an antigen-independent bystander suppression mechanism. Whereas Tr1 and Th3 cells suppress the activation of pathogenic Th1 cells in the local environment via secretion of soluble mediators, the activity of CD4+ CD25+ T cells seems to be dependent on cell-cell interactions, as well. It has been suggested that interaction of CTLA-4 with its ligands is required for the immunosuppressive effects of the CD4+ CD25+ subset (18, 19). This is however controversial (20, 21) and the molecular mechanisms that underly the activity of these cells remain to be determined.

Taken together, various subpopulations of regulatory T cells are involved in the suppression of systemic immune responses, and their presence have been shown to prevent the onset of inflammatory and auto-immune diseases in experimental animal models. However, the

downside of the activity of these cells is the possibility that they may inhibit the induction of anti-tumor responses, thereby interfering with successful tumor rejection, as it has been demonstrated for CD4+ CD25+ T cells in a mouse tumor cell models (22).

### Migration of T lymphocyte subpopulations

The migration of effector and regulatory cell into distinct sites of inflammation is critical for the maintenance of immune homeostasis, for the successful eradication of pathogens with minimal tissue damage, as well as for the prevention of autoimmune diseases. The molecular regulation of lymphocyte migration is complex and involves the interaction of cellular adhesion molecules, such as selectins and integrins, as well as the superfamily of chemokines and their receptors (23, 24).

As a consequence of the distinct and opposite functions of Th1, Th2 and T regulatory cells in the immune response, their migration into sites of inflammation is tightly regulated. Results from a large number of studies have shown that T lymphocyte subpopulations express a restricted pattern of chemokine receptors at their cell surface: Th1 cells preferentially express CXCR3, CXCR6 and CCR5 (25-28), whereas CCR4 (26, 29) and CCR8 (29, 30) are only detected on polarized Th2 cells. CCR3, the first chemokine receptor described to be "Th2 cell-specific" (31) is likely to be expressed on a subpopulation of Th2 cells only. Another cell surface molecule, termed CRTh2 has been described to be specifically expressed on human Th2 lymphocytes in vivo (32). Little information on specific expression of adhesion molecules is available, but levels of the  $\alpha 6 \beta 1$  integrin, which favours migration onto laminin, are higher on Th1 than on Th2 cells (33), suggesting that the differential expression of certain integrins may contribute to the difference in homing capacities observed between Th1 and Th2 cells. At present, no chemokine receptors (34) or homing molecules, specific for Tr1 cells or CD4+ CD25+ regulatory T cells, have been reported.

### Is it possible to phenotypically identify human T lymphocyte subpopulations ?

In addition to their involvement in the functional activity of T lymphocyte subpopulations, differentially expressed cell surface molecules are useful for the identification of these cells. Antibodies against such cell surface molecules might be valuable tools for use in the development and monitoring of clinical therapies, as well as for the isolation of polarized Th populations for research purposes. In addition to chemokine receptors, certain (homologues of) cytokine receptors are differentially expressed on polarized Th1 and Th2 cells. Thus, whereas Th1 cells express both the  $\alpha 1$  and  $\alpha 2$  chains of IL-12 R, no IL-12R  $\alpha 2$  chain is detected on polarized Th2 cells (5). Similarly, expression of IL-18R is detected on Th1, but not on Th2 cells (35).

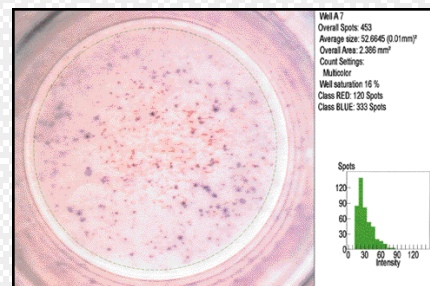
In contrast, Th2, unlike Th1 cells, express a functional IFN-  $\gamma$  R receptor, consisting of both an  $\alpha$  and a  $\beta$  chain (36). Finally, evidence from experimental mouse models suggests that the ST2L molecule, an IL-1R homologue and member of the Toll family of receptors, is expressed exclusively in a stable fashion on polarized Th2 cells (37, 38).

It is important to stress that the expression of many cytokine and chemokine receptors at the surface of T cells is not stable and shows significant variation, depending on the activation state of the cells, as well as on the presence of endogenous cytokines. Moreover, certain cell surface molecules like the IFN-  $\gamma$  R or the IL-12R expressed at very low levels do not allow detection by conventional immunofluorescence and flow cytometry procedures.

It has been argued that cytokine genes are expressed independently from each other and that populations of Th cells form a continuous spectrum in which the Th1 and Th2 phenotypes are only two of the extremes (39). It can therefore not be excluded that cell surface molecules might also be expressed on "intermediate" (Th0) subsets, rendering the identification of polarized Th1 or Th2 cells by analysing the antigens on the membrane, difficult. It should be noted however, that while a single cell surface marker is unlikely to be able to distinguish between the various subpopulations of T effector and regulatory cells, combinations of such differentially expressed molecules might fulfil this purpose.

### ELISA and ELISPOT assays : invaluable tools for cell subset characterization

Future results from genomic approaches, such as transcriptome and DNA array analysis, will draw up a detailed inventory of cell surface proteins and signalling molecules, involved in the function of T helper and T regulatory cells and will provide further understanding of their role in physiological and pathological immune responses. From a practical point of view, since few T lymphocyte subset-specific molecules have been identified, it should be stressed that the operational definition of CD4 + T helper and regulatory lymphocyte subpopulations, based on their different cytokine production profiles, is still crucial for a functional analysis of these cells. Notably, the success of genomic approaches is dependent on the quality of the T cells that are analysed. For this and other Th1/Th2 related events, the detection of cytokine production by ELISA and ELISPOT assays is therefore an invaluable approach.





## References :

- Mosmann, T.R. et al (1986) J. Immunol. 136:234
- Abbas, A.K. et al (1996) Nature 383:787
- O'Garra, G. (1998) Immunity 8:275
- Trinchieri, G. (1995) Annu. Rev. Immunol. 13:251
- Rogge, L. et al (1997) J. Exp. Med. 185 :825
- Robinson, D. et al (1997) Immunity 7:571
- Oppmann, B. et al (2000) Immunity 13:715
- Seder, R.A. and Paul, W.E. (1994) Annu. Rev. Immunol. 12:635
- Groux, H. et al (1997) Nature 389:737
- Maloy, K.J. and Powrie, F. (2001) Nat. Immunol. 2:816
- Chen, Y. et al (1994) Science 265:1237
- Levings, K. et al (2001) J. Exp. Med. 193:1295
- Jonuleit, H. et al (2001) J. Exp. Med. 193:1285
- Dieckman, D. et al (2001) J. Exp. Med. 193:1303
- Stephens, L. et al (2001) Eur. J. Immunol. 31:1247
- Sakaguchi, S. et al (1995) J. Immunol. 155:1151
- Thornton, A. and Shevach, E.M. (1998) J. Exp. Med. 188:287
- Takahashi, T. et al (2000) J. Exp. Med. 192:303
- Read, S. et al (2000) J. Exp. Med. 192:295
- Annacker, O. et al (2001) J. Immunol. 166:3008
- Shevach, E. et al (2001) J. Exp. Med. 193:F41
- Shimizu, J. et al (1999) J. Immunol. 163:5211
- Butcher, E. and Picker, L. (1996) Science 272:272
- Baggiolini, M. et al (1997) Annu. Rev. Immunol. 15:675
- Loetscher, P. et al (1998) Nature 391:344
- Bonecchi, R. et al (1998) J. Exp. Med. 187:129
- Sallusto, F. et al (1998) J. Exp. Med. 187:875
- Kim, et al (2001) J. Clin. Invest. 107:595
- Panina-Bordignon, P. et al (2001) J. Clin. Invest. 107:1357
- Zingoni, A. et al (1998) J. Immunol. 161:547
- Sallusto, F. et al (1997) Science 277:2007
- Nagata, K. et al (1999) J. Immunol. 162:1278
- D'Ambrosio, D. et al (2000) Immunol. Today 21:166
- Sebastiani, S. et al (2001) J. Immunol. 166:996
- Yoshimoto, T. et al (1998) J. Immunol. 161:3400
- Groux, H. et al (1997) J. Immunol. 158:5627
- Xu, D. et al (1998) J. Exp. Med. 187:787
- Löhning, M. et al (1998) Proc. Natl. Acad. Sci. USA 95:6930
- Kelso, A. et al (1995) Immunol. Today 16:37

## Visit our new Website !

**About us**

Diacclone Research catalog contains a large panel of clones starting with a B<sub>2</sub>. The B<sub>2</sub>-stands for hetero, the capital of Franche-Comté, where Diacclone is located and in which laboratories all the B<sub>2</sub>-clones are developed and produced. Over the years these clones have become famous for their quality and excellent performance in flow cytometry (labeled antibodies), biosensors (oxide and endotoxin free antibodies) and detection kits (matched antibody pairs).

The format of the B<sub>2</sub> panel, the line of 200 monoclonal ELISA kits, is available in a wide range with all essential components present in each format to guarantee quality. Parallel to the human cytisine IgG1a, rat IgG1a and a range of mouse cytisine IgG1a are listed.

In the catalogue you can also find a complete range of ELI-spot assays which are designed to measure cytokine or other soluble molecule production at the single cell level. This type of assay enables to detect soluble molecules found only in the microenvironment of producing cells, allowing analysis as close as possible to the in vivo situation. The ELI-spot assay has already shown to be useful to evaluate the vaccination potency of micro-organic vaccines or the infection, or vaccination cycle. Moreover the ELI-spot assay also permits to detect active cytokine producing patterns of Th1/Th2 cells and their shift towards one or the other subpopulation.

All our products are manufactured according to the ISO 9001 guidelines and the non-toxic products, added to the list to serve you with a more complete product line, are coming from a company working under the same conditions.

Diacclone is well known for its research and the production of high quality B<sub>2</sub>-clone monoclonal antibodies (mAb) but our activities and services do not stop there. We also produce gram amounts of mAb in you request under GMP conditions using either your hybridoma cell line or one of Diacclone and produce the mAb in any quantity you want. Developing mAb against your antigen with well-defined characteristics is another service we offer you at competitive prices. Do not hesitate to contact us for these customer services.

Use the search engine if you know which list of products you want or work through. The presented list find four needs for further inquiries.

Besançon, September 2001,  
Dr. John Wolden,  
Director of Diacclone

Tel: +33 30 2001 - You are here - About

The leading orphan drug company in Europe

**Products for your research**

Dear Sir or Madam,

You are looking for a company which can produce gram amounts or more of mAb. We can reply to your demands, our strong points are :

- a pharmaceutical production laboratory with a surface of 300 m<sup>2</sup>,
- many different sized bioreactors,
- a well validated purification protocol,
- and on top of this, the know-how of a professional team.

We are at your disposition for any further information you may need.

Yours sincerely,

Tel: +33 30 2001 - You are here - Introduction

The leading orphan drug company in Europe

**Products for your research**

Antigen section : All

Antigen : All

Reagents : All

Antibody

- ELI-pair 5/36, 10/36, 15/36, 20/36 or 40/36 tests
- ELI-spot agarose gel overlay 5/36, 10/36, 15/36 or 20/36 tests
- ELI-spot complete 1 plate PVDF
- ELI-spot dual color
- ELI-spot matched antibody pair 10/36 tests
- ELI-spot PVDF 5/36, 10/36, 15/36 or 20/36 tests
- ELISA kit 1 plates or 2 plates format
- Quant kit

Keywords

Search Catalogue Number

Search Other products

Tel: +33 30 2001 - You are here - products

The leading orphan drug company in Europe

**HUMAN CD138 ELISA KIT**

REFERENCE : 850.640.096, 850.640.192

SPECIFICITY : Recognizes natural human soluble syndecan-1

RANGE : 8 ng / ml - 256 ng / ml

SENSITIVITY : < 2.56 ng / ml

INCUBATION : 1 h 45 min

SAMPLE TYPES : Serum  
Plasma  
Cell culture supernatant

SAMPLE SIZE : 100 µl

CROSS REACTION : No cross reactivity with other human soluble molecules

KIT SIZE : 96, 192 test

Standard profile

OD	CD138 (pg/ml)
0.0	0
0.5	100
1.0	200
1.5	300
2.0	400



DIACLONE SAS  
1, Bd A. Fleming - BP 1985  
F-25020 BESANÇON Cedex - FRANCE  
Tel. +33 3 81 41 38 38 Fax +33 3 81 41 36 36  
E- mail : info@diacclone.com  
WEB site : http://www.diacclone.com

