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Phenotype and Function of Human T Lymphocyte Subsets: Consensus and Issues

Victor Appay,[1]* Rene A. W. van Lier,[2] Federica Sallusto,[3] Mario Roederer[4]

[1]Cellular Immunology Laboratory, INSERM U543, Avenir Group, Hôpital Pitié-Salpêtrière, Université Pierre et Marie Curie-Paris6, 75013 Paris, France

[2]Department of Experimental Immunology, Academic Medical Center, Amsterdam, Meibergdreef, 15, 1105 AZ, Amsterdam, The Netherlands

[3]Institute for Research in Biomedicine, Via Vincenzo Vela 6, CH-6500 Bellinzona, Switzerland

[4]Vaccine Research Center, National Institutes of Health, Bethesda, Maryland 20892

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*Correspondence to: Victor Appay, Cellular Immunology Laboratory, INSERM U543, Hôpital Pitié-Salpêtrière, Paris, France

Email: appay@chups.jussieu.fr

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• Abstract

In recent years, a tremendous effort has been devoted to the detailed characterization of the phenotype and function of distinct T cell subpopulations in humans, as well as to their pathway(s) of differentiation and role in immune responses. But these studies seem to have generated more questions than definitive answers. To clarify issues related to the function and differentiation of T cell subsets, one session of the MASIR 2008 conference was dedicated to this topic. Several points of consensus and discord were highlighted in the work presented during this session. We provide here an account of these points, including the relative heterogeneity of T cell subpopulations during infections with distinct pathogens, the relationship between phenotypic and functional T cell attributes, and the pathway(s) of T cell differentiation. Finally, we discuss the problems which still limit general agreement. Published 2008 Wiley-Liss, Inc.†

• Key terms

phenotype; function; T cells; virus

THE differentiation of naïve T cells into effector and memory subsets represents one of the most fundamental facets of T cell mediated immunity. Initial descriptions portray effector T lymphocytes as cells found in settings of active antigenic stimulation (e.g., during primary viral infection), able to eliminate viruses or tumors by different effector functions; in contrast, memory T lymphocytes are cells which remain present in the absence of antigenic stimulation and have the capacity to expand rapidly upon secondary challenge (1). However, beyond the apparent simplicity of these original operational definitions lies a complex and controversial classification of an increasing number of T cell subsets. The simultaneous measurement of a variety of surface and intracellular markers enables the distinction between a multitude of T cell subsets (2), particularly in humans. In recent years, a tremendous effort has been devoted to the detailed characterization of the phenotype and function of these distinct T cell subpopulations, as well as their pathway(s) of differentiation and their role in immune response. But these studies seem to have generated more questions than definitive answers, as no general consensus has yet been reached. For the non-expert eye, the multiplicity of reports and the inherent contradictions between models represents an important cause of confusion and even skepticism as to the significance of distinct T cell subsets and their role in immunity. This is counter-productive for developing a unified model of T cell immunity as well as for conducting meaningful and comparable studies.

To clarify issues related to the function and differentiation of T cell subsets, one session of MASIR 2008 was entirely dedicated to this topic. Four experts, who have been working in the field of human T cell immunity, were invited to present their views, opinions, and data on the subject. Rene van Lier pioneered the description of human CD8 T cell subpopulations that could be divided into naïve, memory, and effector subsets based on the expression of cell surface receptors (CD27 and

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CD45RA) (3). Federica Sallusto originally proposed a classification of T cells into central and effector memory (distinguished according to the surface expression of CCR7 and CD45RA), providing an important conceptual advance in terms of T cell dynamics and compartmentalization (4). Victor Appay provided a comprehensive description of virus specific CD8 T cell populations placed along a pathway of differentiation (from early to intermediate to late differentiation) based on the expression of CD27 and CD28 (5). And finally, Mario Roederer pioneered the use of polychromatic flow cytometry, a necessary tool to precisely study the phenotype and understand the function of T cell subsets (6).

Several points of consensus emerged from the work presented by the different speakers, particularly regarding the heterogeneity of T cell subsets and the link between phenotype and function. But a number of issues were also highlighted concerning the pathway of T cell differentiation and the relationship between T cell subset and functional efficacy. Here, we summarize these points of consensus and remaining issues, and draw attention to problems that limit general agreement and the development of a unified model of T cell immunity.

Concurring Opinions

Relative Heterogeneity of T Cell Subpopulations and Relationship Between Phenotypic and Functional Attributes

The T cell population can be divided into distinct subsets based on their phenotype, i.e. the expression of diverse cell surface receptors. The most-commonly used markers are CD45RA (or CD45RO), CCR7, CD27, and CD28. Beyond the precise identification of naive T cells (CD45RA+CCR7+CD27+CD28+), the differential expression of these four molecules allows the distinction between numerous subsets of "resting" (referring to cells that are not involved in primary or acute infection phases) antigen-experienced T cells. The analysis of new cell surface molecules often results in the identification of an increasing number of subpopulations; one may even find as many T cell subpopulations as there are combinations of markers. This could be a reflection of the large heterogeneity of CD4 and CD8 T cell subsets. However, it is essential to notice that the expression of a surprisingly large number of markers significantly overlap or associate with each other (6–24), as summarized in Figure 1. Although such overlap is not strict, general phenotypic profiles or patterns clearly emerge within the heterogeneity of the T cell population. For instance, CCR7+CD45RA−CD27+CD28+ CD8+ T cells, referred to as central memory CD8 T cells, express low levels of molecules like CD57, PD-1, and CX3CR1 but high levels of CD7, IL-7R, and CD62L, in contrast to CCR7−CD45RA+CD27−CD28− CD8 T cells, referred to as highly- or late-differentiated cells. Since the various receptors and molecules analyzed are involved in different cellular functions, general T cell profiles or subsets are associated with a given set of attributes. Several commonly used markers include a range of receptors involved in T cell activation (e.g., CD45RA or CD45RO), costimulation (e.g., CD27 and CD28) or regulation (PD-1). Differences in homeostatic maintenance are implicated by the variation in expression of either cytokines (e.g., IL-2) (3,4) or cytokine receptors (e.g., IL-7R) (20,21). In addition, the expression of some chemokine receptors (e.g., CCR7 and CX3CR1) (4,17) as well as adhesion molecules (e.g., CD11a and CD62L) also seem to follow a common pattern.

Distinctions in effector functions are reflected by the expression of different intracellular molecules. For instance, granzyme K production is a trait of CCR7−CD27+CD28+ CD8 T cells, whereas granzyme B production is predominant in CCR7−CD27−CD28− CD8 T cells (25,26). Distinct CD8 T cell subsets have also been reported to have different capacity to produce cytokines like IL-2 or IFN-γ (Fig. 1) (3). Functionally distinct CD4 T cell populations can also be subdivided according to the expression of chemokine receptors (Fig. 2A). For instance, CCR5 and CXCR3 expression discriminates CD4 T cells with a T_H1 cytokine profile, while CCR3, CCR4, and CRTh2 expression identifies CD4 T cells with a T_H2 cytokine profile (27). CXCR3 and CCR4 are expressed on distinct subsets of CCR7+ central memory CD4 T cells, with CXCR3+ and CCR4+ subsets representing pre-T_H1 and pre-T_H2 cells, respectively (28). Recently, the expression of CCR6 and CCR4 have been reported to characterize a homogeneous population of CD4 T cells that produce IL-17 but not IFN-γ in humans, referred to as T_H17 (29,30). In contrast, expression of CCR6 and CXCR3 identifies a heterogeneous population composed of T_H1 cells and cells producing both IFN-γ and IL-17 (29,30).

It should be noted that the broad correlation between phenotype and cytokine production patterns may not be particularly strong when considering antigen-specific T cell responses. For example, Duvall et al. measured five different functions (IL-2, TNF, IFN-γ, and MIP1β production as well as degranulation) on CD4 and CD8 T cells specific for HIV-2, in concert with the phenotypic markers CD45RO, CD27, and CD57 (31) to identify nearly a dozen phenotypically distinct subsets of T cells. There was remarkably little heterogeneity in the functional profile of these subsets. The strongest associations (with putative differentiation) was the loss of IL-2 production coupled with the gain in MIP1β production and degranulation among CD4 T cell subsets—with little difference in the fraction of HIV-2 specific T cells producing IFN-γ or TNF after stimulation. Within HIV-2 specific CD8 T cells, there was very little difference in the functional capacity when comparing cells across a wide swath of phenotypes.

Different T cell subsets can also be characterized by different lengths of their telomeres, which implies different replicative histories as well as proliferative potentials (19,32,33). Of note, the expression of CD57 on the cell surface shows a strong relationship with telomere length, in that CD57+ T cells have the shortest telomeres (18,32)—implying that these cells have divided the most and have the least proliferative potential amongst the T cell subsets. Overall, the association between distinct subsets and the expression of surface receptors and intracellular molecules implies that these T cell subsets exhibit differential requirements for stimulation and survival, homing potential (e.g., to lymphoid organs or to peripheral tissues) and some (immediate) effector functions.

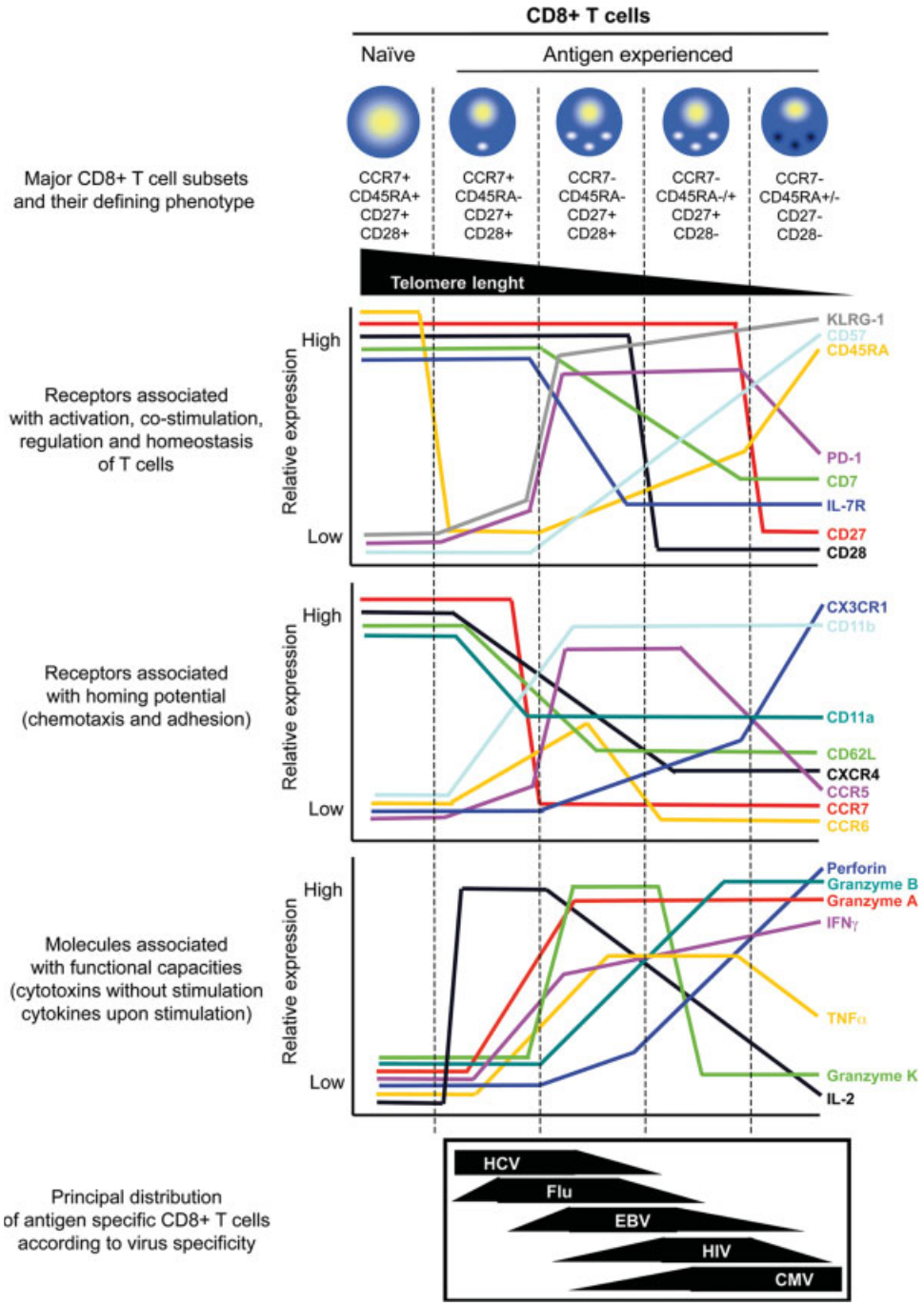

Figure 1. Phenotypic associations within CD8 T cell subsets in humans and relationship with functional attributes. Five distinct subsets of circulating CD8 T cells are defined according to the expression of CD27, CD28, CCR7, and CD45RA. Relative telomere length and expression of a variety of cell surface receptors and intracellular molecules (related to T cell activation, costimulation, regulation, homeostasis, homing potential, and functional capacities) are illustrated in these subsets in a ''resting'' state according to data from the literature. The common phenotypic distribution of virus specific CD8 T cells is also depicted, after clearance of the virus (Flu) or in latent infection stages (for HCV, EBV, HIV, and CMV). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Different Pathogens and Distinct T Cell Response Profiles

Although a common feature of the immunity against infections with pathogens is the central role played by CD4 and CD8 T cells, it has become clear that quite distinct profiles of T cell responses are established for generating memory or maintaining latency to different pathogens. T lymphocytes specific for a virus exhibit some degree of heterogeneity;

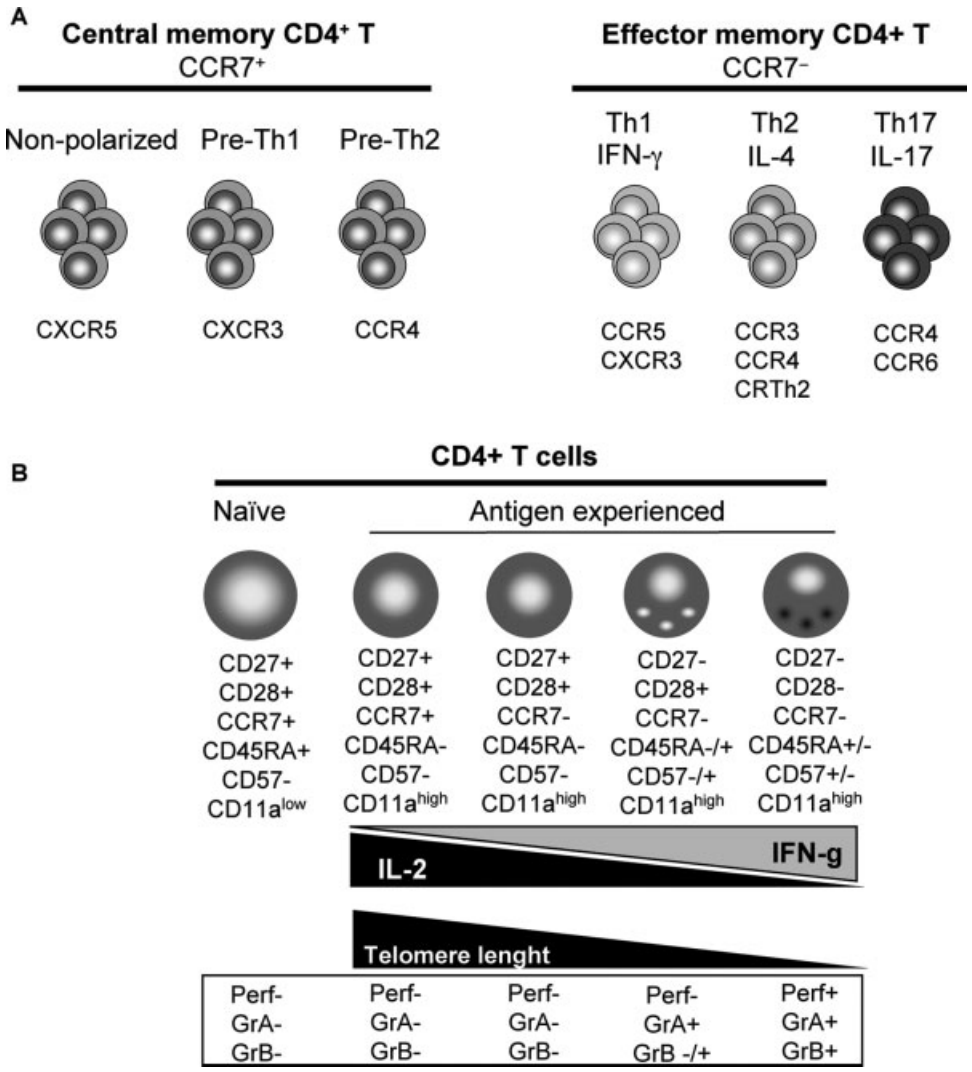

Figure 2. Phenotypic dissection of human CD4 T cells into functionally distinct subsets. (**A**) The expression of chemokine receptors is associated with CD4 T cell subpopulations presenting distinct T_H cytokine profiles. (**B**) The expression of markers, commonly used to define CD8 T cell subsets, enables also the distinction between several CD4 T cell subpopulations, including CD4 cytotoxic T cells.

however, antigen-specific CD8 T cells display unique profiles depending on their viral specificity: Cells are predominantly CCR7+CD27+CD28+, CCR7−CD27+CD28+, CCR7−CD27+CD28− or CCR7−CD27−CD28− during latent infection with HCV, EBV, HIV, or CMV, respectively (5). Following the infection with cleared viruses such as influenza or RSV, virus-specific CD8 T cells appear predominantly CCR7+CD27+CD28+ (34,35). Quite strikingly, the phenotypic profile of CD4 T cells specific for these viruses are also present with very similar distributions (36–40). Although the reasons for the associations between viruses and T cell phenotype remain unclear, a likely role for dictating their profile will be the level and quality of stimulation, which differ between distinct infection settings. Indeed, the antigen load and recurrence, costimulation, and cytokine environment have been shown to influence the phenotype of resulting T cells (32,41,42).

Beyond the T cell phenotype, interesting associations between the functional attributes of CD4 or CD8 T cells and different pathogens have been reported in recent years. In keeping with the observation made on the CD8 T cell phenotype, EBV-specific T cells express high levels of granzyme K but not B, whereas CMV-specific T cells abundantly express granzyme B, but little K (RVL, unpublished data). This latter phenotype is of functional significance as CMV-specific T cells can execute direct cytolysis ex vivo in a granule exocytosis-dependent fashion. Of particular interest, CD4 T cells that lack CD28 expression and display cytolytic potential (43) emerge after primary CMV infection (44). Moreover, in a group of healthy adults, CD4 T cells specific for Candida albicans were found primarily in the CCR6+CCR4+ T_H17 subset whereas CD4 T cells specific for M. tuberculosis were present in the CCR6+CXCR3+ T_H1 subset (29). The elicitation of IL-17 responses correlated with the finding that hyphae of *C.*

albicans primed T_H17 responses in vitro and induced IL-23 but not IL-12 production in human dendritic cells. Most likely, these distinct T cell profiles may reflect differential requirements for effective cellular immune responses required to control different pathogens. Viruses differ in cellular tropism, replication kinetics and other biological properties (e.g., immune evasion mechanisms). The immune system has adapted to these specific traits by mounting T cell responses that appear to be highly adapted to individual pathogens.

The ability to simultaneously measure multiple different T cell functions reveals another level of complexity. Expression of different cytokines is not necessarily correlated; thus, when evaluating the expression of 3 cytokines (e.g., IL-2, TNF, and IFN-γ), each of the seven combinations of coexpression of these three functions can be identified by polyclonal stimulation of T cells. Antigen-specific responses may be restricted to a subset of these combinations. This property of T cells, distinct from their frequency (magnitude of the response) and phenotype has been referred to as the "quality" of the response (45). It is becoming clear that the T cell quality of an antigen response is associated with clinically relevant parameters. The study of this association is still in its infancy; thus, it remains open as to what would comprise the best quality for controlling any given pathogen, and that different pathogens may require different combinations of the optimal types of T cells (different qualities). Nonetheless, a number of studies from successful vaccines, HIV pathogenesis, and protection against parasitic infection demonstrate a common thread: For these instances, "better" quality can be defined as a higher representation of polyfunctional T cells (those cells that simultaneously make all or a majority of measured functions). Specifically, when comparing the HIV-specific T cell response among HIV-infected, clinically defined progressors or long-term nonprogressors, there is a significantly better quality of the CD8 T cell response from the latter group (46,47). Similarly, in individuals infected with HIV-2 (a far less pathogenic virus than HIV-1), the antigen-specific T cells are of better quality than in typical HIV-1 infected adults (31). For viral infections that are well-controlled or cleared (e.g., CMV, vaccinia), the quality of the response is among the most polyfunctional that has been measured (48).

Alteration of T Cell Attributes upon Activation and Inflammation

It is important to note that these observations and relationships between T cell phenotype and functional attributes, as well as between T cell profile and pathogen have been established in the context of resting cells, that is to say, for cells that are not actively stimulated (e.g., by cognate antigen). Many of these observations do not hold in a setting of T cell activation or inflammation. Upon activation, many T cell attributes do indeed change rapidly, such that activated T cells will behave differently from their resting state. For instance, during the acute response to CMV, EBV, and HIV, activated CD8 T cells can be found in the circulating pool that displays a relatively "early" memory phenotype (i.e., CCR7−CD28+CD27+) yet these cells abundantly express granzyme B and are highly cytolytic (5,49). In this respect the differentiation of virus-specific T cells in humans seems to parallel that in mice, in which different memory populations seem to form from a relatively uniform population of acutely expanded effector cells (50). T cell regulation is also dependent on antigenic stimulation, as seen with PD-1, which is upregulated on the surface of CD8 T cells undergoing activation (24), as a likely mechanism to control the proliferation and apoptosis of activated cells (51).

In addition, modulation of chemokine receptor expression occurs upon activation, which implies changes in migratory properties of T cell subsets (52,53). In inflammatory settings, the T cell environment is also altered, which can result in further changes of T cell behavior. For instance, T lymphocytes lacking the lymph node-homing receptors L-selectin and CCR7 do not migrate to lymph nodes in the steady state (4). Nonetheless, studies in the mouse have revealed that inflammatory or reactive lymph nodes (i.e., draining sites of mature dendritic cells) can recruit L-selectin-negative CCR7-T cells. This inflammatory pathway of cell recruitment in lymph nodes requires CXCR3 or CD62P expression on CD8 or CD4 T cells, respectively [FS, unpublished data and (54)]. In reactive lymph nodes, recruited T cells establish interactions with dendritic cells to trigger their maturation (CD4 T cells) or kill them (CD8 T cells). The inducible recruitment of distinct blood-borne T cell subsets to lymph nodes may represent a mechanism for regulating the ability of dendritic cells to activate naive CD4 and CD8 T cells and therefore the immune response.

Inconclusive Points

Differentiation Pathways of CD8 and CD4 T Cell Lineages

Although a number of congruencies with regard to the characterization of T cell subsets have been identified, central points regarding the differentiation and the role of these subsets remain unresolved. The pathway of T cell differentiation, i.e. the sequence of development of the different T cell subsets, remains elusive in humans. Indeed, it is unclear if the differentiation pathway is linear or branched, one-way or reversible. Data on telomere length, which may be one of most informative markers of replicative history, supports a linear pathway of differentiation (see Fig. 1). Moreover, a seminal study in nonhuman primates provides further clues. SIV-infected monkeys were treated with BrdU in order to follow the differentiation of antigen-specific T cells. There was a clear progression of labeled cells, starting from CD28+CCR7+CCR5− ("central memory"), to CD28+CCR7−CCR5− or CD28+CCR7+CCR5+ ("transitional memory"), and then to CD28+CCR7−CCR5+ ("effector memory") T cells; CD28-T cells appeared as terminally-differentiated and represent the end-stage (55).

Following antigen priming, naïve T cells eventually give rise to a heterogeneous population of antigen-specific T cells as seen in vitro (32) and recently in vivo (56); these studies suggest that differentiation is branched and that reversions from one subset to previous can occur. Concerning branched

differentiation, studies in mice have shown that the loss of CD27 expression from activated T cells seems to be specifically induced after interaction with its ligand CD70 (57). In both mice and humans, expression of CD70 is predominantly found on activated immune cells under T_H1 conditions (58). The vast number of CD27− CD4 and CD8 T cells in latent CMV infection might be a reflection of the fact that CMV (re)activation induces upregulation of CD70 (42). However, different pathogens may preferentially induce other costimulatory ligands and/or cytokines which will lead to T cells with different attributes, in a branched differentiation manner.

Along the same line, our understanding of CD4 T cell differentiation remains rather ambiguous. Although CD8 T cells have been extensively characterized, as exemplified in Figure 1, CD4 T cells represent a particular complex population, whose multiple facets have been difficult to grasp. CD4 T cells have originally been divided according to distinct helper functions into T_H1 and T_H2, and lately into T_H17 (Fig. 2A). In recent years, a subpopulation of CD4 T cells has also been shown to have suppressive properties, and are referred to as regulatory T cells (T_{REG}). The expression of markers used to dissect the CD8 T cell population (e.g., CD45RA, CCR7, CD27, CD28), also enable the distinction between several CD4 T cell subsets (Fig. 2B). Using these markers, a number of similarities emerge between CD8 and CD4 T cells, in terms of phenotype, functional attributes, telomere shortening as well as gene expression profiles (43,59,60). Strikingly, the subset of CD4 T cells characterized by a CCR7−CD27−CD28− phenotype has strong cytolytic capacities—a function largely associated with CD8 T cells. The presence of these cells is usually observed in particular settings like CMV or HIV infections (43,44), although their role remains unknown to date. Reconciling these different facets of CD4 T cells and integrating them in a single pathway of differentiation represent major challenges.

Correlation Between T Cell Attributes and Efficacy

An important issue is the absence of association or correlation between the phenotype of T cells and their protective efficacy in vivo. A potential link between failed T cell efficacy and an immature phenotype was initially suggested in that HIV-specific CD8 T cells show a CD27+CCR7−CD45RA− phenotype in contrast to CMV-specific CD8 T cells (characterized by a CD27−CCR7−CD45RA+ phenotype) (61,62) under the assumption that CMV-infection is well-controlled. However, subsequent studies showed no consensus on this theory: HIV-specific CD8 T cells associated with superior control of HIV were also characterized by a CD27+ CCR7−CD45RA− phenotype (47,63); and in the setting of CMV disease, the majority of CMV-specific CD8 T cells displayed also a CD27−CCR7−CD45RA+ phenotype (64). Instead, T cells presenting phenotype characterized with the expression of CCR7 or CD28 were shown to be potentially more efficient at mediating protection in different settings (65–68). Today, the interpretation of phenotypic analysis in relation to T cell efficacy remains ambiguous.

As noted above, polyfunctional T cell representation was positively associated with better clinical outcome (in HIV-1 and HIV-2 infection); similarly, polyfunctional T cells are much more prevalent for viral infections that are completely or well-controlled (vaccinia, CMV). Although these measurements provide a correlate, a recent study of a mouse model of L. major infection demonstrates that the quality of the T cell response is predictive of control following challenge (69). Using a variety of vaccine regimens, mice with similar magnitudes (and phenotypes) of antigen-specific T cells but with dramatically different functional qualities were challenged with live parasites. Mice with the most polyfunctional T cell response showed the best protection whereas those with abundant but monofunctional antigen-specific T cells (producing only IFN-γ) were not protected. This study highlights the importance of functional measurements in determining potential clinical benefit of vaccines or therapeutics. A final aspect of this study showed that the most efficacious T cells (in terms of production), which were the most polyfunctional, produced high levels of cytokine on a per-cell basis. In fact, each polyfunctional T cell produced ~10 times as much IFN-γ as monofunctional T cells (69). Thus, in addition to having the greatest repertoire of functions, these cells are optimized for carrying out those functions. Importantly, these cells were CCR7−: suggesting that the most desirable population to be elicited by a vaccine may be an "effector memory" phenotype population producing IL-2. Given the dogma that CCR7− T cells make little IL-2, this finding underscores the confusion in the literature and the need for a revision of the model.

In summary, there is now a growing body of evidence that the quality of the T cell response is a correlate of protection against viral infection. Although the magnitude and the phenotype of T cell responses will certainly provide additional information about the overall T cell response, these aspects have not yielded any clinical correlates.

Problems Limiting General Understanding

Further understanding of T cell immunity requires that such key issues on differentiation pathways or T cell efficacy should be solved at once. Further work and discussion are therefore needed, based on the completion of comparable and meaningful studies. However, this faces a number of hurdles, some of which are highlighted below.

Need for Consensual Nomenclature

Of utmost importance, there is no harmonization of nomenclature. Even basic terms like "memory," "activated," and "effector" have different meaning in the literature. Memory T cells may be defined as cells that have seen or been primed with an antigen (persistent or not), but one may argue that memory T cells are only found in the absence of antigen. Activated T cells are sometimes referred to as cells that are simply no longer naïve (having been activated at some point by antigen), and are also referred as cells that have been very recently stimulated with an antigen (and, for example, may be entering mitosis). Effector T cells can be cells present during the

primary or secondary phases of an infection, when fighting to halt active viral replication, or they can simply be cells which display effector functions ex vivo (like IFN-γ secretion or perforin content). Much of the confusion is semantic, that is to say related to how these terms are understood and used. Beyond this point, the use of markers to define given subsets also belies some anarchy. For instance, so-called central memory T cells have been defined in the literature, from mice to primates to humans, based on the positive expression of CCR7 or CD62L or even CD27 or CD28. In humans, effector T cells are commonly identified to cells that are CD27− and/or CD45RA+ "revertant", expressing perforin or producing IFN-γ, or expressing low amounts of Bcl-2. Although it is true that some overlap exists between a number of receptors and molecules expressed by a given subset (as seen earlier), this overlap is not strict, and approximation can easily result in inaccuracy, leading to further lack of consensus when comparing studies. This is particularly true when comparing across species, where expression patterns may not be conserved. It is therefore necessary to have reliable definitions for these terms.

Need for Comprehensive Studies and Interpretation

Further efforts need to be devoted to the way studies are conducted and interpreted to avoid short comings. Lack of standardization in experimental procedures and technical differences (e.g., reagents, kinetic of stimulation, fresh versus frozen materials) can be a source of discrepancy in results. In this context, the MASIR meeting offers a strong ground for discussions and validations of state-of-the-art flow cytometry based assays, which is indispensable to deconvolute the complexities of the T cell immune response. For instance, accurate assessment of T cell cytolytic capacity through measurement of perforin production or FATT-CTL assay (see the articles by Betts and coworkers (70) or van Baalen (71), respectively, in the present issue of Cytometry) can only benefit the functional characterization of T cells. Techniques that allow satisfactory detection of subdominant or low avidity CD8+ T cell populations (see the article by Price and coworkers (72) in the present issue of Cytometry) will help us understanding the true role of these cells, which is often overlooked. New insights into CD4+ T cell subset differentiation and function (as proposed by Kern and coworkers (73) in the case of Mycobacterium tuberculosis infection) implies the use of improved methods to identify accurately antigen specific CD4+ T cells; these include the use of MHC Class II tetramers or CD154 analysis (see the articles by Casorati and coworkers (74) or Thiel and coworkers (75), respectively.

Beyond simple technical considerations, there has also been a lack of distinction between activated and resting T cells in most models; however, as mentioned earlier, although these cells may present a similar phenotype, their specific attributes can be significantly different. In humans, longitudinal studies of T cell responses (e.g., from primary infection onwards) are rare (for practical reasons) so that distinct subsets of resting cells are usually compared with each other at a given time point. In contrast, in mice, studies of T cell subsets are mostly performed in a longitudinal manner, and activated (i.e., during primary infection) versus resting (i.e. later) cells are usually compared. This explains why in mouse studies, the definition of effector T cells is mainly related to the timing of their appearance (i.e. during primary infection), whereas in human studies, effector cells are usually thought of more in terms of their functional characteristics (such as their ex vivo cytotoxic potential). It is also important to consider that infections with different pathogens represent different settings (as discussed earlier), for which comparing respective immune responses may not always be as straightforward as it seems. What is observed in one system is not necessarily valid in another system, and different requirements in terms of T cell efficacy may potentially be involved. Last but not least, observations made in the human system do not necessarily apply to the mouse system, and vice versa. It is clear that undue extrapolation and generalization of findings across models and conditions is a problem, which can be a major source of confusion. Full understanding of T cell immunity will require making every effort to perform longitudinal studies in humans, and to take in consideration changes of T cell attributes upon activation and pathogen-specific settings.

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