

## **PREFACE**

Specific immune responses are normally stimulated when an individual is exposed to a foreign antigen [1-4]. The form of immunity that is induced by this process of immunization is called active immunity because the immunized individual plays an active role in responding to the antigen. Specific immunity can also be conferred upon an individual by transferring cells or serum from a specifically immunized individual. The recipient of such an adoptive transfer becomes resistant, or immune, to the particular antigen without ever having been exposed to or having ever responded to that antigen. Therefore, this form of immunity is called passive immunity. Passive immunization is a useful method for conferring resistance rapidly, without having to wait for an active immune response to develop. The technique of adoptive transfer of specific immunity has made it possible to define the various cells and molecules that are responsible for mediating immune responses.

Specific immune responses are classified into two types, humoral immunity and cell-mediated immunity, based on the components of the immune system that mediated the response [5]. Some, called B lymphocytes, respond to foreign antigens by developing into antibody-producing cells, whereas others, called T lymphocytes, are

the mediators of cellular immunity. Humoral immunity is the principal defense mechanism against extracellular microbes and their secreted toxins because antibodies can bind to these and assist in their destruction [6-8]. In contrast, cell-mediated immunity is of critical importance in the defense against influenza virus, some bacterial proliferate inside host cells, and resistance to tumors, and its significance as a role in allograft rejection [9-11]. In some forms of cell-mediated immunity, antigen-specific T cells directly perform the effector function, as when cytotoxic T lymphocytes (CTL) lyse specific target cell [12-14]; in others, antigen-activated T cells secrete cytokines that recruit and activate effector cells that not specific for the antigen, such as nature killer (NK) cells [15], lymphokine-activated killer (LAK) cells [16] and tumor -infiltrating lymphocytes (TIL) cell [17]. When the effector cells are nonspecific, antigen specificity is conferred by proximity to the antigen-stimulated T cells.

Cytotoxic T lymphocytes (CTL) can recognize and respond to foreign antigen only when it is presented in a complex with a self major histocompatibility complex (MHC) molecule on the surface of an appropriate antigen-presenting cells (APC) or target cells [18-20]. Furthermore, specifically sensitized T lymphocytes that are usually CD8<sup>+</sup> and recognize antigens, through the T cell receptor (TCR), on cells of the host infected by viruses or that have become neoplastic [21].

CD8<sup>+</sup> T cell recognition of the target is in the context of MHC class I molecule. Following recognition and binding, death of the target cell occurs a few hours later. This process of differentiation often requires "help" in the form of cytokines secreted by antigen-activated CD4<sup>+</sup> T cells [22-24]. Although CD4<sup>+</sup> helper T cells are not generally cytotoxic to tumors, they may play a role in anti-tumor responses by providing cytokines for effective CTL development [25,26]. In addition, CD4<sup>+</sup> helper T cells that are activated by tumor antigens may secrete tumor necrosis factor (TNF) and interferon- $\gamma$  (IFN- $\gamma$ ), which can increase tumor cell class I MHC expression and sensitivity to lysis by CTL [27]. A minority of tumors that express class II MHC molecules may directly activate tumor-specific CD4<sup>+</sup> helper T cells [28]. In the past few years, considerable evidence has accumulated to suggest the existence of functionally polarized responses by the CD4<sup>+</sup> helper T cell and CD8<sup>+</sup> cytotoxic T cell subsets that depend on the cytokines they produce [29,30]. In general, CD4<sup>+</sup> helper T cells are subdivided into at least two subsets, namely Th1 and Th2 cells, according to distinct cytokine profiles which accounted for two major functions, e.g., cell-mediated immunity and humoral immunity in host immune responses, respectively [26,31]. It is well established that Th1 cells secrete IL-2, IFN- $\gamma$  and tumor necrosis factor- $\beta$  (TNF- $\beta$ ) and function in cell-mediated immunity for protection against intracellular bacteria and

viruses. By contrast, Th2 cells preferentially secrete IL-4, IL-5, IL-10 and IL-13 and provide effective help for B cell responses, in particular for IgG1, IgE and IgA synthesis [32-38]. For the generation of these two subsets of regulatory Th cells, several different cytokines can influence the process of development of Th1 and Th2 cells. For example, IL-12 and IL-4 may direct CD4<sup>+</sup> helper T cell development down a Th1 or Th2 pathway, respectively, while later in development IFN- $\gamma$  and IL-10, IL-4 can reinforce Th1 or Th2 phenotype expansion [39]. Although CD4<sup>+</sup> helper T cells participate in the induction and regulation of cytotoxic T cells, the destruction of the tumor cell is achieved by the CD8<sup>+</sup> cytotoxic T cell with specificity for the antigens on the surface of the tumor cells.

Nature killer (NK) cells constitute phenotypically and functionally a diverse population of large granular lymphocytes (LGL) in peripheral blood and spleen [15]. For example, these cells typically show the morphology of large granular lymphocytes (LGL) and express CD16 and CD56 (human) or NK-1.1/2.1 (mice), but do not express CD3 and do not rearrange or express Ig or TCR genes [15]. NK cells are spontaneously cytotoxic against a variety of target cells, including certain tumor cell lines and virus-infected cells as well as allogeneic bone marrow (BM) and lymphoid cells [40,41]. Unlike CTL, this activity was initially considered to be non-specific and non-MHC restricted, NK

cells are capable to discriminate specifically between their targets [42]. Although NK cell specificity involves both activating and inhibitory receptors, in general a target inhibition type of activation mechanism determines this specificity [43,44]. Several reports suggest that NK target engagement could be inversely correlated to the expression of specific MHC class I molecules on the targets [45,46].

These observations have been strengthened by the identification of the MHC class I-specific Ly49 C-type lectin family of inhibitory receptors on mouse NK [47,48], and KIRs (killer cell inhibitory receptors) on human NK cells [49,50]. Furthermore, recent evidence suggests that the NK-activating stimuli, delivered by target cells, could be overruled by class I-mediated inhibition [51]. Thus, it is conceivable that NK cells may play a role in immunosurveillance against developing tumors, especially those expressing viral antigens.

On the other hand, lymphokine-activated killer (LAK) cells are derived from normal or tumor patients cultured in medium with recombinant IL-2 *in vitro* [52,53]. LAK cells also show non-MHC restricted cytotoxicity similar to that of NK cells, but are able to kill a broader of targets [54]. It is now widely accepted that the majority of IL-2 activated killer (LAK) cells are derived from the large granular lymphocyte (LGL) natural killer (NK) subset of lymphocytes with a CD3<sup>-</sup> CD16<sup>+</sup> CD56<sup>+</sup> phenotype. These cytotoxic cells have demonstrated

broad lytic reactivity against a large variety of tumor cell lines and fresh, surgically-obtained human tumors [55]. This property has been exploited in the clinic for the treatment of cancer by immunotherapy with IL-2 and/or LAK cell [56]. In addition to their tumoricidal activity, it is found that IL-2 induced human LAK cells can also acquire the ability to lyse autologous and allogenic monocytes in vitro [57]. Furthermore, mononuclear cells derived tumors, called tumor-infiltrating lymphocyte (TIL), also include CTL with the capacity to lyse the tumor from which they were derived [17,58].

They are cultured with high concentrations of IL-2, leading to expansion of these activated T lymphocytes in vitro [59]. TIL is very effective in destroying tumor cells and have proven much more effective than LAK cells in experimental models [60]. These studies showed that TIL has 50 to 100 times the antitumor activity produced by LAK cell. It is thought that TIL is also necessary to optimize antitumor effects of TIL delivered to the tumor as a part of adoptive immunotherapy.

Lymphocytes such as NK cells, LAK cells, TIL and CTL are considered to be useful for adoptive immunotherapy to treat tumors and both induction and expansion of these lymphocytes have been investigated extensively [53,61-63]. In particular, CTL have shown higher cytotoxicity than LAK cells and TIL against specific tumors that

have been used as antigen-like stimulators during induction of proliferation [64,65]. Therefore, CTL have been suggested as effective killer cells in adoptive immunotherapy for tumor cells [66,67].

Due to the need for tumor cells or tumor-derived antigens to be present as a continuous stimulation for induction and proliferation of CTL, recent studies have focused mainly on identification of tumor antigens and preliminary therapy for tumors [68,69]. This is presumably because a system for reproducible generation of human autologous CTL has not been established yet, as neither established autologous tumor cells lines nor tumor-derived antigenic peptides for repeated stimulation of the lymphocytes are available. To replace autologous tumor cells, autologous tumor-specific CTL generated using HLA-matched tumor cells [70,71] or peptide-pulsed antigen-presenting cells (APC) [72-74] have been developed. Antigen-presenting cells (APCs) serves as an immunological window to the foreign world.

In general, the various types of APCs can be divided in to professional and non-professional cells. while the latter are found among nonlymphoid cell, professional APCs, such as macrophage ( $M\Phi$ ), dendritic cell (DC) and B cell, form an intergral part of the immune system [75,76]. However, although they show common characteristics in their ability to ingest, process and present antigens, these cells serve very different immune functions. Since all T cell-mediated immune

responses require antigen presentation, APCs may be of central importance for the generation and regulation of tumor immunity.

Soluble proteins and synthetic peptides are generally poor immunogens for in vitro generation of MHC class I-restricted CD8<sup>+</sup> CTL. In fact, generation of in vitro CTL responses is usually dependent on in vivo priming, either through natural infection or deliberate vaccination. However, recent studies have demonstrated that primary CTL responses may be induced in vitro through stimulation with peptide-pulsed mutant APCs such as RMA-S or T2 cells lines which are deficient in TAP genes [77-79]. It is generally believed that the capacity of these cells to induce primary, peptide-specific CTL responses in vitro is due to high density of relevant peptide/class I complexes on the surface of the APCs [80]. It is recently also shown that DC and M $\Phi$  can take up exogenous antigenic proteins or peptides in vitro and induce an antigen-specific CTL response.

In this study, we have generated HLA-A2402-restricted, antigen-specific CTL response by primary in vitro stimulation in a culture system using autologous peripheral blood cells from both healthy donors and unimmunized carcinoma patients. Carriers of HLA-A24 comprise approximately 60% of the Japanese population and 98% of them have the HLA-A2402 subtype. These results suggest that a suitable APC type and a method allowing efficient loading of exogenous

antigenic proteins or peptides may be important for in vitro induction of primary CTL, an important step towards CTL adoptive immunotherapy.