Chapter 8
An Ancestral Immune Surveillance System in the Amphibian *Xenopus* Connecting Certain Heat Shock Proteins with Classical and Nonclassical MHC Class I Molecules

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Abstract Studies in the amphibian *Xenopus*, a vertebrate species that diverged from a common ancestor with mouse and human more than 350 million years ago, provide evolutionary insights into the convergent roles of certain hsps such as gp96 and HSP70 as well as classical and nonclassical MHC class I molecules in cancer immune surveillance. Evidence that in *Xenopus* gp96 and HSP70 can elicit potent antitumor responses dependent on antigen representation by nonclassical MHC class Ib molecules and presumably involving innate T cells suggests the existence of an ancestral immune surveillance system in antigen-presenting cells such as macrophages integrating hsps with classical and nonclassical MHC molecules. The particular connection revealed in *Xenopus* between hsps and nonclassical MHC molecules presenting conserved patterns to innate T cells affords new avenues to develop therapeutic strategies against cancer.

Keywords Comparative immunology · Innate T cells · Tumor immunity · Evolution · Unconventional T cells

8.1 Introduction

Heat shock proteins (hsps) are evolutionarily ancient and highly conserved molecular chaperones constituting several multigenic families that are produced by all cell types and perform essential biological functions under normal as well as stressful physiological conditions [1]. Some of these hsps including gp96 (a member of the hsp90 family) and the cytosolic 70 kDa hsps or HSP70 (defining indistinctively

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both the inducible hsp72 and the constitutively expressed hsc73) have received a lot of attention because of their potential use in tumor immunotherapy (reviewed in [2–4]). HSP70 and gp96 have been shown to elicit potent CD8 T-cell responses specific against the antigenic peptides they chaperone not only in humans and mice [5–7] but also in frogs [8, 9]. These hsp-mediated CD8 T-cell responses are MHC class I restricted and depend on the internalization of the hsp-antigen complexes by endocytic receptors such as the α2-macroglobulin receptor CD91 at the surface of antigen-presenting cells (APCs; [10, 11]). This is followed by the representation of chaperoned antigenic peptides by MHC class Ia molecules on APCs to CD8 T cells [7, 12, 13]. The functional connection between hsp chaperoning and MHC class I antigen presentation may have even further ramifications than previously thought considering that in addition to classical MHC class Ia (class Ia) a growing number of nonclassical MHC class Ib (class Ib) and class I-like gene have been characterized (reviewed in [14, 15]). Some of these class Ib genes encode proteins that are hypothesized to be indicators of intracellular stress and malignancy (reviewed in [16, 17]. The potential role of these class Ib molecules is of particular relevance in immune surveillance and recognition of aggressive class Ia low or negative tumor cells through their interaction with T-cell receptors and/or non-T-cell inhibitory or triggering receptors expressed by NK and unconventional T cells.

Focusing on two of the most conserved hsps, gp96 and hsp70, studies in the amphibian *Xenopus* have provided compelling evidence that the immunological properties of these molecular chaperones, especially their significant antitumor responses, have been conserved during evolution (Reviewed in [18]. Comparably, while nonclassical MHC class Ib genes in *Xenopus* do not share a direct common ancestor with their mammalian counterparts, some of these genes encode molecules with striking analogous functions including class Ib-restricted unconventional T-cell-mediated antitumor immune responses.

We review here recent advances using the amphibian *Xenopus* to explore the potential of an ancestral immune surveillance system composed of hsps such as gp96 and hsp70, endocytic receptors such as CD91 and classical and nonclassical MHC class I molecules.

8.2 The *Xenopus* Immune System

The immune system of the South African clawed frog *Xenopus laevis* exhibits all the basic elements of jawed vertebrate immunity. The primary immune organs thymus and spleen and adaptive B- and T-cell effectors expressing a wide Ig and TCR repertoire generated by RAG-mediated somatic diversification as well as innate cell effectors such as neutrophils and macrophages are all conserved in *Xenopus* (reviewed in [19]). In fact, the fully sequenced and annotated genomes of two different *Xenopus* species, *X. tropicalis* and *X. laevis*, have provided compelling

evidence of the remarkably high degree of overall conservation of immune genes between *Xenopus* and human.

One intriguing aspect of anuran amphibians such as *Xenopus* that is not encountered in mammals is that the development of the immune system occurs at two distinct times: first during larval life and then again during the metamorphic transition from tadpole to adult [20, 21]. Specifically, the *Xenopus* thymus is first colonized by embryonic stem cells a few days after fertilization [22]. During metamorphosis, the thymus loses about 90% of its lymphocytes [23]. This loss is followed by a second wave of stem cell immigration [24, 25]. The tadpole is free-swimming and amenable to a variety of surgical (e.g., thymectomy, transplantation) and nonsurgical (e.g., adoptive transfer of leucocytes, injection of hormones, antibodies) interventions. Therefore, studies in *Xenopus* tadpoles can be helpful in collecting valuable information otherwise difficult to gather from in utero studies in mammals (e.g., development of self-tolerance to adult-specific antigens, acquisition of a second T-cell repertoire, and ontogeny of T-cell subsets in a natural setting).

A second aspect of *Xenopus* immunology that makes it attractive as a model is the absence of classical MHC class Ia protein expression in tadpoles until the onset of metamorphosis. Surface class Ia expression is first detected on erythrocytes and on splenic leukocyte populations at pro-metamorphic stages [21, 26, 27]. Although tadpoles are immunocompetent and have CD8 T cells, the larval thymus lacks significant expression of class Ia and LMP7 genes until metamorphosis, which suggests an inefficient class Ia-restricted T-cell education during larval life [21, 28]. Conversely, multiple class Ib genes are expressed by thymocytes at the onset of thymic organogenesis consistent with a role of class Ib molecules in early T-cell development.

Thus, the high degree of functional conservation of the *Xenopus* immune system with human, the natural class Ia-deficient tadpole stages, as well as the amenability of *Xenopus* to in vivo experimentation make it a highly relevant nonmammalian model (reviewed in [19, 29]). In particular, *Xenopus* is well suited to study tumor immune surveillance and as such has proven instrumental to exploring innovative approaches for cancer immunotherapy (reviewed in [19, 30]).

8.3 Lymphoid Tumors and Tumor Immunity in *Xenopus*

X. laevis is the only amphibian species in which a series of true lymphoid tumor cell lines have been derived and characterized from spontaneously occurring thymic tumors ([31, 32]. Two similar thymic tumors were also reported at the *Xenopus* colony at Tulane University around the same time [33]. More recently, another type of spontaneous leukocytic, possibly monocytic, tumor very different from the thymic tumors originally characterized was described [34].

Importantly, the occurrence of spontaneous thymic tumors in MHC-defined inbred and *X. laevis/X. gilli* isogenetic clones has provided a unique opportunity to derive

lymphoid tumor lines growing in in vitro culture as well as in vivo following transplantation in compatible *X. laevis* host [32, 35]. From the partially inbred F strain homozygous of the *f* MHC haplotype, two different tumor lines (B3B7 and ff-2) were derived, whereas from the isogenetic clone LG-15 heterozygous for the MHC haplotype a/c, 15/0 and 15/40 lines were obtained. These cell lines are all nonadherent and grow continuously at 27 °C with a generation time of 18–24 h [36]. All four tumor lines share a mixed immature T-/B-cell phenotype: they all express several pan T-cell markers such as CD8 and CD5 but have also rearranged their Ig gene loci. All the tumor cell lines also express the cortical thymocyte-specific Xenopus cell surface marker (CTX), a marker of immature thymocytes that in the organism is only expressed by cortical thymocytes [37, 38]. Another salient feature exhibited by all these tumor lines is the expression of high level expression of several *Xenopus* nonclassical MHC class Ib (*XNC*) genes, including XNC1, 4, 10, and 11 as well as β2-microglobulin [39]. In contrast, only the ff-2 tumor expresses low levels of classical MHC class Ia at the cell surface, whereas 15/0, 15/40, and B3B7 cell lines are all class Ia-negative [32, 35].

Two of these lymphoid tumor cell lines have remained transplantable in compatible hosts. The ff-2 tumor is transplantable in the MHC homozygous *f/f* partially inbred F strain, whereas the 15/0 can grow in the isogenetic LG-15 background. Interestingly, the ff-2 tumor line is tumorigenic when transplanted into F tadpoles but not into F adults. The rejection of ff-2 tumor in F adults is abrogated by γ-irradiation that preferentially depletes thymocytes and is impaired in T-cell-deficient thymectomized animals, which suggests the critical involvement of adult T cells that differentiate just after metamorphosis [35, 40]. Comparably, the 15/0 tumor cells are highly tumorigenic when transplanted into both tadpole and adult LG-15 hosts [32, 35]. In addition, the 15/0 tumor line is transplantable and tumorigenic in another isogenetic clone, LG-6, that shares the same MHC haplotypes (a/c) with LG-15 animals but differs at multiple minor histocompatibility (H) loci [41]. This difference in minor H-antigens has been instrumental in exploring antigen-specific antitumor immunity in *Xenopus* as delineated in the next chapter.

Initial in vivo and in vitro studies have revealed that in *X. laevis* as in mammals NK and CD8 T cells are critical antitumor cell effectors [41]. Briefly, the involvement of NK cells was demonstrated by anti-NK antibody treatment followed by tumor transplantation assays and by an in vitro cytotoxic assay [41–43]. Thymectomy at early developmental stage before cell precursor immigration and sublethal γ-irradiation that mainly affect dividing thymocytes and circulating T cell provided evidence of CD8 T cells requirement to control malignancy [35, 40, 44]. Importantly, taking advantage of the absence of class Ia expression by 15/0 tumor cells has allowed us to shed light on the unappreciated roles of nonclassical MHC class Ib molecules and unconventional class Ib-restricted T cell in *X. laevis* tumor immunity (see Chap. 5).

8.4 Conservation of Antitumor Properties of Heat Shock Proteins

The *X. laevis* tumor immunity model has provided evolutionary evidence of the ability of certain hsps such as the endoplasmic resident gp96 and the cytosolic HSP70 to elicit potent antitumor protective T-cell responses. In mammals, these molecules can induce pro-inflammatory cytokines, stimulate NK cells, and elicit potent cytotoxic CD8 T-cell responses against the antigenic peptides they chaperone [2–4]. The representation of antigens chaperoned by these hsps in the context of MHC class Ia by APCs critically involves the endocytic receptor CD91 [10, 11] as well as other scavenger receptors [45–47]. The additional interaction of these hsps with various signalling receptors such as TLRs is associated with their ability to stimulate inflammation [48, 49].

Given the high degree of evolutionarily conservation of gp96 and hsp70 across vertebrate and even invertebrate species, it was of interest to determine whether the immunostimulatory properties of these hsps, especially regarding antitumor immunity, were also conserved in amphibians such as *Xenopus*. Using minor H-Ags differences between LG-15 and LG-6 cloned frogs, it was first demonstrated that, as in mouse and human, both gp96 and hsp70 were able to represent chaperoned minor H-Ags and generate efficient CD8 T-cell responses recognizing and killing targets expressing the same minor H-Ags in a MHC-restricted fashion [8]. Immunization by direct subcutaneous injection of hsp70 or gp96 chaperoning minor H-Ags as well as by adoptive transfer of macrophages pulsed with hsp70/gp96-minor H-Ag complexes was shown to generate immunological memory to minor H-Ags leading to accelerated rejection of minor H-Ag-matched skin grafts [8, 50]. As in mammals, *Xenopus* gp96 and HSP70 can interact with the endocytic receptor CD91 at the surface of APCs, which leads to its rapid internalization and the representation of its bound antigens by MHC class Ia [51]. These studies in *Xenopus* strongly suggest that certain hsps (gp96, HSP70) and hsp receptors (CD91) are all integral parts of an ancestral system of immune surveillance. The importance of this system in controlling neoplasia is highlighted by its conservation for more than the 350 million years that separate amphibian and mammals from their common ancestor.

Furthermore, since, in contrast to skin grafts, the 15/0 lymphoid tumor does not express class Ia molecules, our comparative tumor immunity model has permitted investigation of the potential roles of hsps in stimulating MHC class Ia-unrestricted NK and unconventional T cells in the context of antitumor immunity. Both in vivo and in vitro studies demonstrated that immune responses against 15/0 tumor cells in *X. laevis* involve NK cells and unconventional classical class Ia-unrestricted CD8 cytotoxic T cells (CCU-CTLs) that both were shown to kill 15/0 tumor cells but not class Ia expressing non-tumoral lymphoblast targets in vitro [41]. The critical involvement of chaperoned antigens in hsp-mediated anti-15/0 tumor immune responses in the absence of class Ia presentation is supported by several lines of evidence. For both gp96 and hsp70, native forms purified from non-tumoral organs (e.g., liver) or recombinant forms

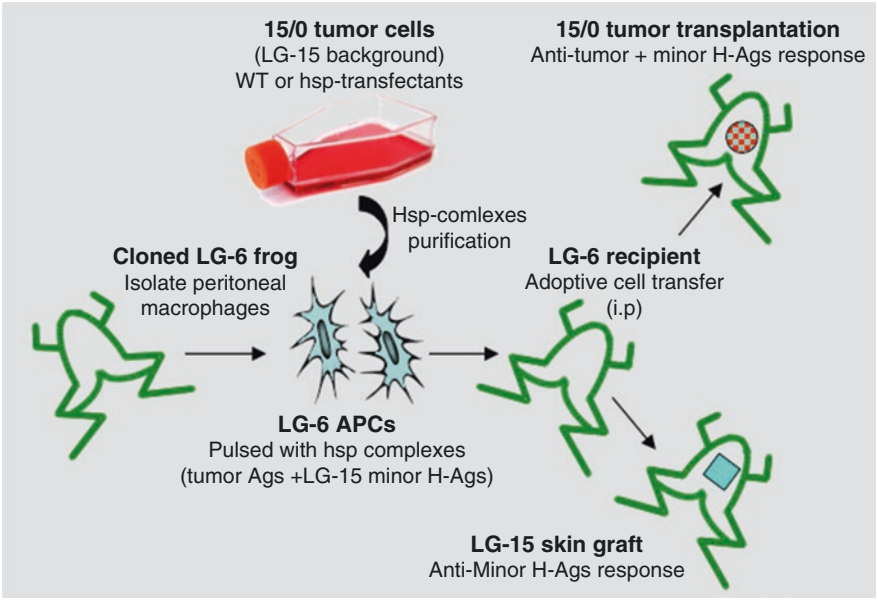

Fig. 8.1 Schematic of the antigen representation assay developed in *Xenopus*. Peritoneal macrophages elicited by stimulation with heat-killed *E. coli* are recovered from LG-6 adults by peritoneal lavage and used as APCs. Hsps are purified from 15/0 tumor WT or stable transfectant expressing tagged recombinant *Xenopus* hsps. Since 15/0 tumor is on the LG-15 background, hsps chaperone both minor H and tumor Ags. LG-6 macrophages are pulsed for 1 h on ice with the hsp complexes at a concentration of 0.5–1 mg per 1×10^5 cells, extensively washed, and then adoptively transferred into LG-6 recipients (5×10^5 cells per animal). Hsp-mediated immune responses elicited against minor H-Ags can be monitored in vivo by monitoring the rejection time of minor H-disparate LG-15 skin graft. Hsp-mediated antitumor immune response can be monitored by determining the time of tumor appearance following injection of 15/0 tumors

produced from bacteria or non-15/0 cells (e.g., B3B7 cells) did not elicit significant anti-15/0 tumor immune response and the removal of ligands from hsp70 by ADP abrogated anti-15/0 immunogenicity [9, 50].

To specifically address MHC class Ia-dependent and class Ia-independent antigen representation, we developed an in vivo adoptive cell transfer assay using *X. laevis* peritoneal macrophage (pMac) as APCs that is depicted in Fig. 8.1. First, we demonstrated that adoptive transfer of pMac exposed to either gp96- or hsp70-minor H-Ags complexes generated a CD8 T-cell response specifically against minor H-skin Ags and that this response was dependent on the endocytic receptor CD91 [51]. We then showed that a similar but class Ia-independent representation of hsp chaperoned antigens was involved in the case of the anti-15/0 tumor immune response [50]. Accordingly, LG-6 pMac exposed to tumor-derived gp96 and adoptively transferred into LG-6 hosts markedly impaired the growth of transplanted 15/0 tumor in a CD91-dependent manner.

In the case of hsp70, we went further to distinguish the respective role of the inducible hsp72 and the cognate or constitutively expressed hsc73. Although these two types of cytosolic hsp70 share very similar primary structure, they exhibit significant differences in their peptide- or ligand-binding domains, subcellular localization, and some of their function [52]. To be able to examine the tumor immunogenicity of each hsp70 isoform, we produced *X. laevis* recombinant cognate hsc73 and the inducible hsp72 from stable 15/0 tumor transfectants. Both hsp72 and hsc73-Ag complexes exhibited a similar ability for eliciting class Ia-mediated T-cell responses against minor H-Ag skin grafts. In contrast, our in vivo representation assay revealed that hsp72 was more potent than hsc73 in generating protective immune responses against the class Ia-negative 15/0 tumors in an Ag-dependent and putatively class Ib-mediated manner. This study provided the first evidence that although hsc73 is as potent as hsp72 in facilitating class Ia-restricted T-cell responses, it is less efficient than hsp72 in eliciting class Ia-unrestricted antitumor T-cell responses that are class Ib-mediated.

8.5 Conserved Roles of Nonclassical MHC and Innate T Cells in Tumor Immunity

As a method of immune evasion, tumors often downregulate their class Ia expression and thus facilitate their escape from conventional T-cell-mediated immune recognition and killing [53]. Importantly, loss of class Ia expression constitutes a loss of "self-signal" and can subsequently render malignant cells more susceptible to NK cell-mediated cytotoxicity. Consequentially, in order to avoid NK-mediated killing, many different types of tumors induce or upregulate the expression of class Ib genes [16]. Accordingly, an increased expression of certain class Ib molecules has been postulated to be an indicator of malignancy and/or intracellular stress [16]. Although the critical implication of classical MHC class Ia in tumor immune surveillance by eliciting effective antitumor CD8 cytotoxic T-cell effectors is well established from *Xenopus* to mammals, the roles of nonclassical MHC class Ib molecules and the effectors interacting with these molecules from NK to unconventional and innate T cells are less well understood.

The functional relevance of class Ib molecules in the cancer field is still unclear and often contradictory. Clinical studies have confirmed class Ib upregulated expression as a hallmark of certain tumors and shown that this typically correlates with unfavorable prognostics. HLA-E and HLA-G, in particular, have been shown to be indicators of poor clinical outcome in several different types of cancer [54–58]. On the other hand, other class Ib proteins, both in human and mouse, have been credited with the ability to mediate protective immunity against a variety of different cancers. In fact, due to their critical regulatory roles in immunity, certain class Ib molecules have emerged as attractive therapeutic targets against malignant neoplastic growths [59, 60]. Among potential class Ib targets, CD1d is perhaps the most

studied. CD1d is critical for the development and function of CD1d-restricted invariant natural killer T-cells (iNKT) cells, which despite their relatively small numbers play critical regulatory roles promoting antitumor responses [59–61]. Several ongoing clinical trials are evaluating the effect of CD1d-mediated stimulation of iNKT cells with α-galactosylceramide (α-GalCer) on cancer patients (reviewed in [62]). Even though no clear tumor regression was observed, the iNKT-based therapies increased INF-γ blood levels, provided disease stabilization, and prolonged mean survival in patients no longer responding to chemo- or radiotherapies.

However, efficient clinical implementation of CD1 and iNKT cell-based therapies is still far from realization and requires a deeper and comprehensive understanding of the biology of this system.

From an evolutionary perspective, both class Ia and class Ib genes have been found in all jawed vertebrates studied to date (reviewed in [63]). Although relationships between evolutionarily distant class Ib molecules are difficult to establish, functional analogs, such as the primate HLA-E and the mouse Qa-1b, have been identified [64]. Representatives of the CD1 family of genes are found in mammals [65, 66], birds [67, 68], and reptiles [69] but in neither fish nor amphibians. In *X. laevis* there are at least 23 class Ib (*XNCs*) genes that, like other vertebrate class Ibs, are heterogeneous, less polymorphic, and less ubiquitously expressed than class Ia [39, 70–72]. Many of these *XNC* genes have an unusually high degree of conservation between *X. laevis* and *X. tropicalis* species both in primary sequence and genomic organization [70, 72]. The strong gene selection maintained in these two *Xenopus* species that diverged from a common ancestor as long ago as primates and rodents (~65 million years; [73]), is in support of important biological functions of *XNC* genes.

In this context, the high expression levels of several *XNC* genes by tumor lines derived from several independent lymphoid thymic tumors take on particular relevance. The possible involvement of certain *XNC* genes and XNC-restricted innate T cells in tumorigenesis and antitumor immunity in connection with hsps are all exciting avenues of investigation offered by the *Xenopus* model. To begin elucidating the functions of these *XNCs* in our tumor immunity model, we have chosen a loss-of-function reverse genetic approach based on RNA interference to silence *XNCs* at the level of the tumor. More specifically, the relevance of these *XNCs* for 15/0 tumorigenicity was investigated both indirectly by silencing *b2m*, which is usually required for surface expression of MHC class I molecules including class Ibs, and directly by silencing the expression of multiple *XNC* genes by targeting a consensus sequence shared by most *XNC* transcripts [74]. In fact in the case of *XNC10*, we were able to show the requirement of *b2m* surface expression. Interestingly, both types of silencing resulted in comparable results. 15/0 tumor transfectants deficient in either *b2m* or *XNCs* expression were more susceptible to NK-mediated killing but more resistant to killing by CD8 T cells in vitro. Moreover, 15/0 tumor transfectants were more tumorigenic in vivo upon transplantation in LG-15 adult recipients [74]. The faster tumor development of these *XNC*- or *b2m*-deficient tumor transfectants despite their decreased resistance to NK cell killing in vitro further suggested an

important involvement of unconventional T cells interacting with XNC molecules rather than being restricted by MHC class Ia molecules.

However, further elucidation of the role of distinctive *XNC* gene products in this tumor model has revealed this to be more complex than previously thought. XNC10 represented an ideal candidate to focus on, since it is among the highest *XNC* expressed in 15/0 tumor and it is conserved, not only in *X. laevis* and *tropicalis* but also across ten different *Xenopus* species. Intriguingly, the specific silencing of *XNC10* in 15/0 tumor resulted in an acute rejection of these tumor transfectants by syngeneic LG-15 adults as well as naturally class Ia-deficient LG-15 tadpoles [75]. In tadpoles, the rejection was more potent toward 15/0 tumor transfectants with stronger *XNC10* knockdown. Furthermore, the rejection of XNC10-deficient tumors implicated cell-mediated cytotoxicity that could be enhanced by priming [75]. As such, *XNC10* is necessary for the immune evasion of the thymic-derived 15/0 tumors to escape immune recognition and class Ia-independent cytotoxicity. Taken together these findings suggest that various XNC molecules have different and possibly even opposing roles in immune surveillance, underlining the critical roles of class Ib molecules in tumor immunity. It is possible that different XNCs interact with distinct effector cells resulting in a balance between inhibitory and activating signals leading to either increased or decreased tumorigenicity.

8.6 Conserved Roles of Class Ib-Restricted Innate T Cell in Antitumor Immunity

Among MHC class Ib-restricted effector cells, innate T (iT) cells such as CD1d-restricted iNKT cells have recently emerged as a potentially critical component of tumor immunity as they can orchestrate both innate and adaptive immunity [76–79]. These lymphocytes are T cells with natural killer cell markers and expressing semi-invariant T-cell receptor (TCR) repertoires [14]. Although iT cells generally occur at low frequencies [80], they can control immune responses via rapid and potent release of either pro-inflammatory or anti-inflammatory cytokines [81].

Notably, we have recently demonstrated that iT cells are not only conserved in *Xenopus*, but may constitute a more prominent component of their immune system than in mammals, especially during tadpole life [82]. To date we have been able to characterize the iT cell subset restricted by XNC10 [15, 82]. Using a reverse genetic approach combining transgenesis with RNA interference, we showed that XNC10 is required for the development of these iT cells. Furthermore, based on TCR diversity, XNC10 tetramer binding, and CD8 antibody staining, two subpopulations have been characterized within the *Xenopus* XNC10-restricted iT cells, type I XNC10-T^{+}/CD8− and XNC10-$T^{dim+}CD8^{dim+}$, which are reminiscent of mammalian type I iNKT and type II NKT cells, respectively [82].

Interestingly, rapid infiltration of XNC10-iT cells is observed following intraperitoneal 15/0 tumor transplantation into LG-15 tadpoles [75]. Similar early

infiltration of XNC10-iT cells also occurs when transplanting ff-2 tumor into inbred F tadpoles (Banach and Robert, unpublished observations). Intriguingly, knockdown of XNC10 in 15/0 tumor triggers a substantially increased infiltration of XNC10-iT cells, which is again consistent with the use of XNC10 as an immune evasion strategy by the 15/0 tumors.

8.7 Conclusions and Perspective

Antigen presentation by classical MHC class Ia molecules as a way to induce potent antigen-specific CD8 T-cell responses is a pivotal component of the immune surveillance system. More specifically, in the context of tumor immune surveillance, APCs are postulated to acquire tumor antigens generated by deregulated gene expression and/or mutations from the malignant cell and then generate an adaptive T-cell response specific to these antigens. Hsps, such as cytosolic HSP70, and ER-resident gp96 can contribute to elicit this antitumor response by chaperoning tumor antigens thus facilitating efficient cross-presentation as well as by enhancing the co-stimulation responses important for potent activation of T cells.

Here, we propose that hsps, classical MHC class Ia, nonclassical MHC class Ib molecules, and their respective effector cells are integrated in an ancestral immune surveillance system (Fig. 8.2). Indeed, the critical involvement of class Ib molecules

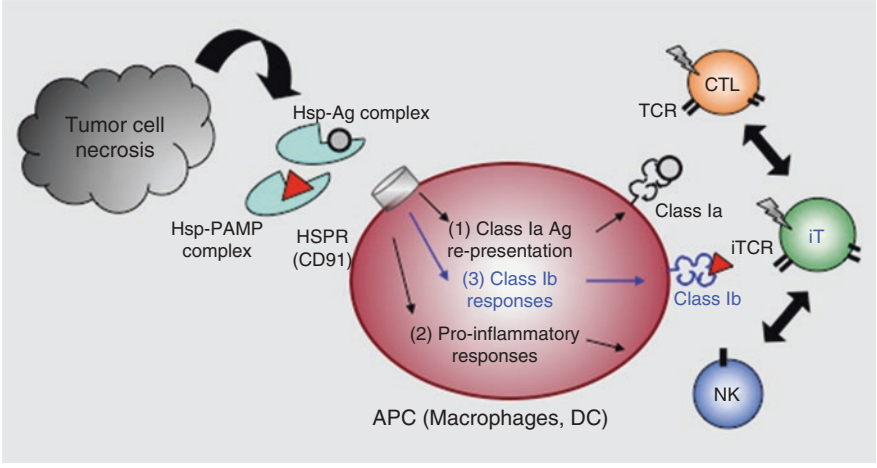

Fig. 8.2 Proposed ancestral immune surveillance system. Hsp-peptide complexes released in the extracellular compartment from infected or stressed cells (e.g., apoptosis, cell lysis) are internalized by APCs through receptor-mediated endocytosis (e.g., CD91). (1) Antigenic peptides channeled into the class Ia presentation pathway activate CD8 T cells. (2) Hsps internalized by the same receptors or interacting with other receptors (e.g., TLRs) stimulate pro-inflammatory responses. (3) Hsps are proposed to also stimulate class Ib-mediated responses by an as yet unknown mechanism that is likely to be Ag-specific and involve iT cell populations

in amphibian hsp-mediated antitumor responses and the finding that class Ib-restricted antitumor iT cells are present and prominent outside mammals raise the intriguing possibility that this system is ancestral and widespread across jawed vertebrates. Although the role of nonclassical MHC molecules and unconventional T cells, including iT cells in tumor immunity, is still far from fully elucidated, the inherent ability of class Ib molecules to present nonprotein antigens such as lipids and other conserved molecular motifs or patterns offers an extended avenue of detectable antitumor determinants. The limited variation of these class Ib-binding patterns and their conservation during evolution could be exploited as target of choice for future immunotherapy. In addition, the potent and rapid activation of unconventional class Ia-unrestricted T cells such as iT cells may be critical in promoting antitumor versus pro-tumor suppressive microenvironments.

In this context, the ability of hsps to also promote iT cell responses through class Ib molecules is a promising new avenue to investigate. Given that during tumor progression class Ia molecules are often downregulated, cancer immunotherapies that exploit class Ia-restricted T-cell effectors are usually insufficient to maintain potent antitumor responses. Conversely, as some class Ib molecules remain expressed on tumors or in some cases are even upregulated, these molecules and their interacting immune effector cells could serve as additional persisting immunogenic targets. Thus, the elucidation of the roles of class Ib molecules in tumor immunity is of fundamental scientific and clinical interest.

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