

Today, when one picks up a modern immunology text book or review (1-3), it will often present an incorrect listing of the characteristics of the several intrinsic „antigen types“ that are reported to exist on human and animal cancer cells or do not consider the autochthonous immunogenicity of the various non-self altered components of cancer cells resulting from the oncogenic process. Most significantly these reviews almost never consider the distinction between those cell components which serve as true immunogens as defined above for the tumor-bearing host and those cancer cell components which lack such autochthonous or autologous immunogenicity and are only markers detected with polyclonal antibodies made in a different species. Further, if the 37 kD oncofetal antigen / immature laminin receptor (37kD OFA/iLRP) (4-15) we have reported to be expressed on all mammalian tumors we have tested to date is considered at all as a tumor immunogen, it is usually

Cancer Vaccine Technology Update: The Immunobiology of 37 kDa Oncofetal Antigen/ Immature Laminin Receptor Protein (OFA/iLRP) and RNA, a Universal Tumor Immunogen

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tumor-bearing host and this important trait is rarely if ever considered in the classification of these cellular components associated with cell growth regulation or differentiation.

Hence, we have approached this lexical problem several times in prominent journals and reviews to no avail (5,6,13-15). We suggest this is a critical mistake and is not restricted to a few recalcitrant tumor immunobiologists but extends to experienced reviewers and editors who rarely challenge this erroneous classification, even in the latest texts and

reviews (1-3). This is yet another effort to deal with the peer-reviewed, widely published facts related to autologous tumor immunogenicity to hopefully correct these long-standing misconceptions so that at least new immunology textbooks and scientific articles will hopefully present something closer to reality for their readers.

● Tumor Associated Antigens vs Tumor Rejection Immunogens:

True tumor rejection antigens or immunogens (TRAs or TRIs) {our term preference} must first and foremost be recognized as foreign (non-self) proteins to the extent that these proteins arouse detectable T-cell mediated immune responses directed against the auto-immunogenic protein(s) expressed on the emerging cancer in the host. To be effective in arousing a protective, host T-cell mediated „Adaptive Response“ which is capable of destroying the tumor cells, this immune response to any TRA's present on a cancer cell must activate CD4⁺ helper T cells (T_{H1}) and CD8⁺ effector cytotoxic T-cells (T_c) stimulated by CD83⁺ dendritic cells (DC)

or other antigen processing cells (APCs) (7-11). A cardinal feature of the adaptive immune response against tumors is its „exquisite specificity of T cells {T_c cells} for distinct macromolecules and 'memory', which is the ability to respond vigorously to repeated exposures“ to a non-self immunogen present on cancer cells (1).

True tumor rejection inducing



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incorrectly clustered with the carcinoembryonic antigen (CEA) and the alpha fetoprotein (AFP) in spite of our past protests (6,13,14). The lack of autochthonous immunogenicity by AFP, CEA and other tumor markers like cellular oncogene products (cONCs) is a fundamental property of AFP and CEA differentiation markers in the



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immunogens (TRIs) thus, must be capable of activating T_C-mediated immunity in the autologous host. Autochthonous antibody induction against TRIs is widely reported to play at least a minor role in tumor rejection (7). The use of the term „antigen“ to characterize non-immunogenic markers like CEA, AFP and c Oncs, which appear on normal as well as cancer cells must be avoided. If the protein is not immunogenic in the primary cancer-bearing host and must be detected with antibody made in a foreign species (most correctly called a „xenoantigen“), such a protein is not a true TRA for the cancer-bearing host but simply „markers“ to denote cell replication and differentiation. This is a fundamental requirement to differentiate normal cell components from tumor-specific components that activate tumor rejection. Markers are usually expressed in greater concentration on tumor cells than normal cells and are never tumor specific, since they are also expressed on normal adult cells and tissues.

Thus, the second criterion that must be determined to warrant the status of being ranked as a tumor rejection immunogen (TRA) is that the cancer-associated protein or other component must not be expressed immunogenically by normal adult tissues of the host. However CTL responses to melanoma differentiation antigens such as MelanA/Mart-1 can induce CTL responses to tissue specific antigens and destruction of melanocytes resulting in vitiligo. Moreover, neurological paraneoplastic syndromes can be due to spontaneously induced immune responses against tumor cells, which, however, cross-react with neuronal components. The bottom line in this terminology distinction (Antigen vs. Immunogen) is that the historical term „tumor antigen“, as reflected in the immunological literature, does not automatically imply its **immunogenicity** in the autochthonous host. Unfortunately, most modern reviews of tumor immunology historically do not make this important distinction (1-3). The seeds of this confusion in terminology rest in a classic dogma that has allowed certain proteins expressed on normal diploid cells of an animal or human to be classed as „Tumor Antigens“ even if they lack immunogenicity in the host with the cancer. Such misnomers give the reader the

impression that these „antigens“ are true tumor „immunogens“ for the host when, in reality, they are only present in higher concentrations on tumor cells than normal cells of the host, but, in fact, these proteins typically lack the ability to activate host T-lymphocytes which can kill the homologous tumor cells. Likewise, the cancer-bearing host is unable to make a T cell response to these self-macromolecule.

Normal adult tissues of a human generally lack any immunogenic determinant(s) which can arouse a host T or B cell mediated response due to *deletion of precursor thymic cells* which can „recognize“ self antigens during maturation in the normal thymus or inactivation of those that escape deletion. If this were not so, mammals would have tremendous auto-immunity problems. However the efficiency of negative selection in the thymus drops with aging so autoreactive T cells get out of the thymus and nonspecific stimulation of these T cells via bacterial products (through APC) can induce or exacerbate autoimmune diseases. Usually however, they don't cause a problem because of either immunologic ignorance or because of T regulatory cell activities. Furthermore, a lot of these autoreactive T cells are probably inactivated because they get no costimulation signal even when they do interact with an autoantigen on a normal cell (due to lack of CD80/86 expression by most cells). Hence, the most important so-called „tumor rejection-capable“ immunogens likely to be useful in creating an effective and specific anti-cancer vaccine or be useful to detect malignant cancers are those few TRAs which are absent from normal adult tissues and are processed as T-cell immunogens in the cancer-bearing host. Some, but a very few, human cells located in immunologically „privileged sites“ (ie., anterior chamber of the eye, testis and central nervous system) may express an autoimmunogenic „self-antigen(s)“ in the host, but such cells are restricted to the sequestered anatomical site. Cancer/testis (CT) antigens are examples of tumor components normally expressed and restricted to male germ cells in the testis but are not present in adult somatic tissues. Spontaneous humoral and cell-mediated immune responses have been demonstrated against several CT antigens, including NY-ESO-1, MAGE-A, and

SSX antigens (16)

● Immune Responses against Tumors

All manner of studies seeking to identify and characterize TRAs have been reported since the 1950's when serious studies were first attempted (17-19). In the years prior to 1950 much that was assumed to be true about the characteristics of cancer immunobiology was defined in outbred rodents. Potent T-cell activating antigens detected to be present in such non-syngeneic model tumor systems were, in fact, indistinguishable from MHC determinants present on the tumor cells; hence, the rejection data generated was valuable only in defining histoincompatibility determinants, not tumor-specific autoimmunogens (17). Later studies were conducted with inbred mice, hamsters and rats and clarified this problem and mandated that tumor rejection antigens (TRAs) had to be identified in syngeneic rodents (18-20). Since the term „antigen“ generally implied any protein that was observed to elicit an immune response in a test host, the term became loosely used in the cancer immunology literature. Subsequently, genuine tumor-specific rejection immunogens (TRAs) were detected to be present on a variety of cancer types in syngeneic or autochthonous hosts. Viral-encoded immunogens (v Oncs) expressed on virus-induced neoplasms (21), authentic OncoFetal Antigen (OFA) (4-6,11,13,14,22,23), mutated p53 proteins in human cancers (24) or mutated proteins chaperoned to the cell surface membrane are examples of true TRAs/TRI's.

The major development in the first 20 years of studying tumor associated immunogens/ antigens in inbred animals was the concept of „**Immune Surveillance**“ (reviewed recently in 25,26). Three lines of clinical / pathological evidence that the human or other mammalian host immune systems can be aroused against TRAs appearing on tumors and not normal tissues are:

Lymphocytic infiltrates developing around primary tumors contained activated, immunogen-specific T-cells in immunocompetent syngeneic hosts (27-29).

T-cell lymphocytes are routinely activated against tumors in inbred experimental animals and humans by direct vaccination with syngeneic tumor

cells from another syngeneic animal or in the autologous patient, respectively, or were detected subsequently following successful tumor removal and elimination by surgery. The immunity mediated by these antigen-specific T cells can passively transfer immunity (adoptive immunity) to naive, inbred, syngeneic rodents when the animals receiving the immune T-cells are subsequently challenged with syngeneic tumor cells expressing the tumor-specific immunogen. Control, non-immune mice receiving adoptive transfer of non-immune T-cells are not similarly resistant to the same challenge with a variety of tumor cells including carcinomas, leukemias or sarcomas (30-32).

T-cell-deficient humans and animals experience an increased incidence of certain types of cancer like lymphoma/leukemia (33,34).

● **Types of Tumor Antigens [Note: Not all these are true TRAs]**

1. Mutated self-proteins which are rendered **immunogenic** to the tumor-bearing host. Chemical carcinogens, radiation-induced or spontaneous mutations in cellular genes encoding these altered proteins all express these individually specific, immunogenic proteins which are *unique to each tumor clone*. These unique immunogens are termed tumor specific transplantation-like antigens „TSTAs“. TSTA's are *unshared* with other tumors even when other primary tumors induced by the same chemical or radiation carcinogen appear on the same host animal (35-37).

2. Cellular encoded Oncogene proteins (c Onc). These **non-immunogenic**, normal cell proteins are linked to normal regulation of growth properties of the cell and are generally over-expressed by tumor cells (eg., abl gene) (38). This over-expression is frequently due to altered mutations induced by carcinogens in one of several cellular encoded Tumor Suppressor genes (eg., p-53 protein) which produce proteins which regulate the cONC expression levels by normal cells. Cellular or cONC genes may also be translocated on the chromosome during cell transformation to alter their expression levels, but additional control mechanisms have been observed to impact their expression levels (39,40).

3. Oncogenic viruses encoded transforming gene products (v ONCs) introduced by abortive infection of

host cells with tumor-producing viruses. Examples are: Human papilloma virus E6 protein in cancerous warts, E7 protein in cervical carcinoma (41,42); EBNA proteins in EBV-infected cells (43), SV40 or Adenovirus encoded T-antigen proteins in various human cancers induced by these viruses (44) including human papovaviruses like BK or JC polyoma viruses. Such v ONCs are classically shared **immunogenic** proteins to the host when expressed in viral-transformed human and animal cancer cells by a given virus like SV40 T-antigen. These v ONC products may also be expressed on non-cancer cells infected permissively with these oncogenic viruses but such cells are routinely lysed or damaged when producing infectious oncogenic virus.

4. Over-expressed differentiation associated antigens (DAs) or non-mutated self-proteins (eg., CEA (45,46), AFP (47-50), HER-2/neu (51), human telomerase reverse transcriptase (hTERT) (52,53), tyrosinase (54), gp100 (55,56), tyrosinase-related protein (TRP)-1 (57), TRP-2 (58), etc.) are non-immunogenic markers present in the primary, tumor-bearing host and are routinely expressed on normal tissues in adults.

5. Heat Shock Protein-Peptides Complexes (HSP-PCs): HSPs (gp96, hsp70, hsp90, calreticulin, hsp 110 and grp 170) are a family of inducible but also ubiquitously and constitutively expressed self-derived protein chaperones involved in assisting protein folding and unfolding in the cells. Because of their ability to chaperone cellular peptides, HSPs, derived from animals with cancers, elicit protective immunity specific for the cancer from which the HSPs are purified. This property allows CTL activation without the need to identify the corresponding antigen and provides the basis for a new type of cancer vaccine. HSPs-chaperoned peptides can be presented to CTL by DCs in the context of MHC class I molecules through receptor-mediated endocytosis. CD91 (also known as $\alpha 2$ macroglobulin receptor) has been recently identified as one of these receptors (reviewed in (59,60)). In a pilot study, 16 patients with different types of cancer were vaccinated with autologous tumor-derived gp96, which is known to be immunogenic in mice (61). Six of 12 patients that could be tested developed MHC class I-restricted tumor-specific T cell response (62). A similar study

performed on metastatic melanoma patients receiving autologous tumor-derived gp96-peptide complexes led to an increase in specific T-cell responses against melanoma antigens in 48% of patients and to clinical responses in 18% of patients (63).

6. True Oncofetal Antigen (37kDa OFA/iLRP). This protein is immunogenically re-expressed on **all mammalian cancers examined to date**, yet remains undetected and rendered non-immunogenic when expressed in term fetus, neonate tissues, and in the inbred and outbred pregnant host's adult tissues due to dimerization, acylation and other maturational changes (4-6). 37 kDa OFA/iLRP is a **shared, universal** tumor rejection immunogen (TRA)(5). OFA/iLRP can also induce immunoregulatory CD8+ T suppressor cells (iLRP-specific T cells secreting IL-10) which disables Tc/CTLs directed against any tumor rejection antigen (TRAs including OFA/iLRP) (7-11) described in a subsequent section.

7. Unmasked Mucin proteins. The mucin family of glycoproteins is characterized by a variable number of amino acid tandem repeats and extensive O-glycosylation at serine and threonine residues. To date, at least eight mucin genes, encoding the protein backbone of these glycoproteins have been identified. One of these genes, MUC1, codes for the membrane-bound mucin (64). In some carcinomas, MUC1 has frequently been found to have increased, unpolarized expression as well as under-glycosylation, allowing for greater immunogenicity by revealing otherwise masked epitopes in its protein core (65). Such unmasked MUC1 proteins are **immunogenic** when processed endogenously by host APCs (66,67).

These correctly classified tumor associated macromolecules are listed in **Table 1** and their immunogenicity in autochthonous hosts are noted.

● **Host CTL or Tc responses**

Shared OFA or unshared, tumor clone unique TSTAs are capable of arousing the required CTL or Tc responses that can attack and kill emerging tumor cells *in vivo*. Both types of immunogens are present on rodent and human cancer cells (5-11,13-15,23). TRAs are endogenously synthesized cytosolic proteins which are displayed at the tumor or early fetal cell surfaces in conjunction with class I MHC associ-

ated proteins on early transforming as well as fully-transformed malignant cells or embryo cells or early fetal cells. These antigens are recognized by class I MHC-restricted CD8⁺ CTLs whose function is to attach to and subsequently kill tumor cells expressing the immunogen. CD8⁺ CTLs are detected in rodent tumor challenge models where tumors are induced by chemicals, radiation or oncogenic viruses (e.g., vONCs -mutated, normal cell proteins, etc) (5-11,13-15,23).

CTLs are activated against tumors by recognition of MHC-bound peptides of tumor-specific immunogens like viral encoded v ONCs or cellular-encoded TSTAs and by universally expressed OFA/iLRP on the surface of host antigen-presenting DCs or other professional antigen-presenting cells (APC's) which ingest tumor cells or their antigens and present these antigens to two T cell subclasses (ie., CD4⁺ helper T lymphocytes (Th1) and naive CD8⁺ precursor T lymphocytes) (5-11,13-15,23). The CD8 precursor cells differentiate into tumor antigen-specific CD8⁺ CTLs or Tc's. The CD8⁺ T -cell activation is promoted by APC interaction of co-stimulator B7 determinants present on the APCs

that develop then can kill tumor cells expressing the class I MHC-bound homologous tumor immunogen peptide without either the co-stimulator or CD4 Th1 helper interaction.

Thus, CTLs are induced by cross-presentation of a tumor immunogen by host APCs and are then effective in killing the tumor cells. Some immunologists have reported that Th1 helper cells can kill tumor cells to a limited extent directly (71,72) but, if true, this is very limited killing. Antibodies to some tumor antigens which require Th2 helper cell interaction have also been implicated in tumor cell killing, but this too results in marginal killing if it occurs. Likewise, activated macrophages and natural killer cells (NK) have been linked to the killing of some tumor cell types *in vitro*, but the role of such anti-tumor antigen reactivity has not been effectively established to operate significantly *in vivo*.

It is understandable that many investigators have incorrectly deduced that primary tumors which may display one or several potential tumor immunogens can only induce „weak“ tumor resistance (19,20,73-75). Such immunity is presumably easily overwhelmed by challenge with high levels of tumor

or „ineffective antigens“ (73,75,76). We have suggested that this view reflects an incorrect assumption (5,6,13-15) because the investigators claiming such weak immunogenicity did not recognize that **immunoregulatory mechanisms** involving a newly discovered subclass of CD8 T-cells called **T-suppressor cells** can be activated by a tumor immunogen such as OFA/iLRP (7-11).

When OFA/iLRP immunogen-specific Ts cells are activated as the predominant subclass in animals responding to these new immunogenic macromolecules, the OFA-activated Ts lymphocyte subclass may be down-regulating the tumor-bearing host's T-cell mediated immunity in the presence of high levels of certain tumor specific immunogens (7-11). Since this is often true, the concept of „weak“ TRIs becomes erroneous and misleading. In addition, some tumors may also utilize several other immunologic „tricks“ that prevent maximal impact of the T -cell immune system on the developing cancer. Some examples are tumor cell secretion of immunosuppressive cytokines such as TGF-β and IL-10, loss of MHC determinants needed for effector T-cells to recognize tumor T cell immunogens, and mutation or

Table 1: Tumor Associated Immunogens and Marker Types Expressed on Human and Animal Reprinted with the permission of Anti-Cancer Research (100).

Name	Immunogenic in Cancer-Bearing Host	Example
Mutated Cellular Self-Proteins ¹	+	TSTA
Cellular Oncogene Products [c Onc] ²	--	abl
Viral-Induced Oncogene Products [v Onc] ³	+	SV40 polyoma; ADV T-antigen
Differentiation Antigens [Das] ⁴	--	CEA, AFP
Heat Shock Protein-Peptides Complexes ⁵	+	Gp96-chaperoned peptides
Oncofetal Immunogen ⁶	+	37kD OFA/iLRP
Unmasked Mucin Immunogen ⁷	+	MUC-1

with Tc-expressed CD28 subsequent to Tc T cell receptor binding of class I MHC-bound tumor antigen peptide. Class II MHC: tumor antigen peptide-activated CD4⁺ Th 1 cells promote differentiation of the CD8⁺ precursor Tc-cell into fully activated tumor specific CD8⁺ CTLs by cytokine secretion. Presentation of processed tumor antigen to both class I and class II MHC-restricted, tumor antigen-specific T cells by dendritic cells which took up tumor antigen is called „Cross-priming or Cross-presentation“ (68-70). The activated tumor-specific, effector CTLs

cells (5,6,13-15). Since emerging cancer cells actually express several *de novo* immunogenic macromolecules in the host, all these immunogenic proteins could be considered „weak“ antigens or immunogens in the host since primary tumors emerged successfully in the immune-competent animal, yet the host developed cytotoxic T-cells against these immunogens. In other words, all these immunogens produced an ineffective host T-cell response to eliminate the immunogenic tumor. The concept that unfortunately emerged was that all tumor rejection immunogens are „weak“

reduced expression of the immunogenic tumor protein. The latter may be of importance because tumor antigens expressed at levels sufficient for cross-presentation by bone marrow-derived stromal cells appears to overcome immunological „ignorance“ to solid tumors (77). Some researchers reported that tumors of humans and animals are weakly immunogenic. Increasing the immune reaction may have little effect or may actually stimulate rather than inhibit the growths of these tumors in their primary host. (74,78). This too is incorrect (7-11)!

How does OFA/iLRP differ from CEA, and AFP and other so-called carcinoembryonic proteins?

As stated above, CEA, and AFP are „Differentiation Antigens“ (DAs), and are not tumor rejection immunogens (TRAs). Only 37kDa OFA/iLRP actually warrants the designation „Oncofetal Antigen“. Sir Peter Medawar and the first author of this article (JHC) coined the term OFA in the early 1970s (14,22). These key criteria were that **true OFA** is only expressed in **immunogenic form** in the developing mammalian embryo and early fetus during pregnancy and is re-expressed in all malignant tumors of mammals tested by us and others to date (5,6,13-15). Most important, OFA/iLRP is not expressed in **immunogenic form** in normal adult and /or normal regenerating cells (see details below). In contrast, CEA, and AFP are not immunogenic in the pregnant female or in the cancer-bearing host and **are present** on many, but not all normal, regenerating cells of several organs. True OFA ceases to be synthesized as an immunogen for the pregnant host in late term fetus and in normal adult animals after birth due to the maturational process to mature LRP previously described.

Background Summary of OFA/iLRP: Immature laminin receptor protein (iLRP) is a 37kD, monomeric, non-acylated, true oncofetal immunogen in the strictest sense (4,5). It is **immunogenic** for the mother being actively expressed on the surface of cells comprising part of the embryo and early fetus in utero - even in inbred pregnancy when expressed in early embryo and early fetus (5,6,15,23). In contrast, **mature 67 kD** laminin receptor protein (mLRP) is rendered **non-immunogenic** before birth of the mature fetus because it is dimerized and acylated to *mature LRP* (mLRP) in the newborn and is often linked non-covalently to β -galactin (4). Mature LRP is expressed at varying levels on some normal adult cells which function in the adult host to enable these differentiated cells to egress through laminin basement membranes in normal tissues and vessel walls. That is, OFA/iLRP, the 37 kD immature fetal-restricted molecule, unlike mLRP, is a true „immunogen“. Following early neoplastic cell transformation, OFA/iLRP is re-expressed universally on cancers induced by viral, carcinogen

and radiation-induced cancers as well as spontaneous cancer cells arising from other mutations in DNA in the host. OFA/iLRP is never expressed on any normal or differentiating cell in the neonate, juvenile or adult mammal. Mature and immature LRP both attach to laminin (4).

To be a true **autologous immunogen** (capable of inducing an immune response in the adult host) a protein must be recognized as foreign to its own thousands of other so-called **self-proteins** which comprise that animal's normal tissues and cells. For example, AFP and CEA are expressed on normal dividing adult liver cells and a few other organspecific cells in the animal at birth and on normal fetuses of that animal (4-11). When the normal adult liver makes new, normal cells to replace damaged liver, and other cells in the adult animal from undifferentiated normal liver precursor cells that give rise to replacement liver cells, they classically express lots of AFP as do diploid fetal liver cells. When these precursor cells are fully differentiated into normal liver cells, they continue to express only small amounts of the AFP. AFP is over-expressed on several types of normal adult cells during normal cell replication and is generally present in high levels in some *tumor clones*, but it is never normally immunogenic for that host.

The host mammal expressing DAs is usually immunologically incapable of recognizing DAs as *non-self* proteins. Why? T lymphocyte precursors undergo a process called „thymic maturation“. During thymic maturation, precursor T cells (thymocytes) that develop T cell antigen receptors which bind self antigens presented in the thymus too well **are deleted** by apoptosis to render the host mostly incapable of responding to its own tissue thereby preventing autoimmune disease. Most importantly, human CEA, and AFP, unlike OFA/iLRP, are most easily detected with antibodies made in a foreign host like a sheep or goat. Antibodies against OFA/iLRP can be induced in autologous tumor bearing hosts or by immunizing inbred mice with syngeneic early fetal cells expressing OFA/iLRP (23)

CD8 T lymphocytes cloned from spleens of either MCA1315 fibrosarcoma-bearing BALB/c mice or from BALB/c mice immunized with

different doses of recombinant OFA/iLRP were shown to be H-2 K^d-restricted in the recognition of different OFA/iLRP epitopes (79). Also, although both OFA/iLRP-specific cytotoxic T cell (CTL) clones and IL-10-secreting, non-cytotoxic CD8 T (Ts) cell clones recognized OFA/iLRP peptide epitopes that shared similar protein topology, the epitopes recognized by the cytotoxic CD8 T cells were distinct from those recognized by the IL-10-secreting, non-cytotoxic T cells (79). Also, immunization of BALB/c mice with syngeneic dendritic cells which had been pulsed with a cocktail of OFA/iLRP peptides that contained epitopes that activated OFA/iLRP-specific CTL, but not OFA/iLRP-specific Ts cells, induced spleen CD8 T cells which secreted predominantly γ -interferon, but not IL-10 upon stimulation with intact OFA/iLRP. However, if the spleens came from BALB/c mice immunized with syngeneic dendritic cells that had been pulsed with OFA/iLRP peptides that contain epitopes that stimulate IL-10-secreting CD8 Ts cells, but not CTL, the predominant cytokine secreted by the immune spleen CD8 T cells in response to OFA/iLRP is IL-10 (79). Thus, immunization with certain OFA/iLRP epitopes should potentiate anti-tumor effector cell immunity and so potentiate immunotherapy effects.

● Self-antigens and Tumor Immunotherapy Trials

To date, the large majority of the known human tumor „antigens“ used in cancer immunotherapy are non-mutated self proteins which are over-expressed by tumors (80). However, some researchers still find them to be promising for the purpose of diagnosis and immunotherapy. This may explain the confusion in the field between an „antigen“ and an „immunogen“ because any promising antigen is immediately used as an immunogen in clinical immunotherapy trials in an attempt to control tumor growth. The apparent success in these trials depends on the following:

1. Despite the thymus being exposed to these proteins during embryonic development, some peptide-specific T cells probably escape deletion during the ontogeny of the immune system. Activation of these T cell clones is achieved by presenting these epitopes in

an immunostimulatory context, such as by engineered dendritic cells (50). DCs when transduced with a recombinant adenovirus (Adv) vector encoding e.g. AFP, will process and present epitopes in the context of MHC class I antigens and can induce AFP-specific protection (47,50). Similarly, DCs pulsed with agonist CEA peptide epitopes induce CEAspecific effector T cells and block thyroid carcinoma progression (81,82). Also immunization of CEA-expressing cancer patients with a canary poxvirus vector encoding CEA and B7.1 has produced disease stabilization and increased CEA-specific effector T cells in 37% of patients in a small clinical trial (81).

2. However, most of these trials are based on the use of high affinity dominant epitopes as targets for specific CTL responses (53,83-85). Despite some positive results, there is increasing evidence to indicate their clear lack of efficacy, probably due to tolerance to dominant determinants on self-antigens (84,86). Breaking tolerance to dominant epitopes on these self-antigens is, indeed, becoming one of the major goals of tumor immunologists in the preparation of vaccines (86-88). To circumvent immunologic tolerance to dominant epitopes, cryptic epitopes are used for tumor immunotherapy provided that they are efficiently presented by tumor cells (30,89,90).

3. The use of high-affinity heteroclitic variants of low affinity epitopes to mobilize CTL targeting low affinity epitopes provided that they are presented by tumor cells efficiently enough to be recognized by CTL. However, low level of expression of low affinity epitopes should be a barrier for their use in immunotherapy, since CTL effectors require a small number of peptide /HLA complexes on the target surface to be activated (91).

However, the use of the non-mutated self-antigens in tumor immunotherapy raises the risk of autoimmunity, due to the expression of these antigens on normal cells, including the thymus. This could be harmless if the antigen is present in an immunoprivileged organ, shielded from an autoimmune response, as in the case of MAGE-A-expressing testis but in other cases could be a great problem, like in the hTERT, which is expressed in activated T and B cells and in the CD34⁺ hematopoietic progenitors (92).

● Summary of Current Results using OFA/iLRP in Human Cancer Systems

1. Breast Carcinoma Patients.

While all breast carcinomas tested have expressed OFA/iLRP (8), it was important to determine if breast carcinoma patients made effector T cell responses to OFA/iLRP on their tumor cells. We found that the peripheral blood T lymphocytes of the breast carcinoma patients we studied proliferated abundantly to autologous breast carcinoma cells, but not to other patients' breast carcinoma cells. This proliferation was MHC restricted (8). From these tumor-induced proliferating T cells, we were able to clone both CD4 and CD8 T cells which proliferated specifically to autologous breast carcinoma cells. All of these autologous breast carcinoma-reactive T cell clones were CD3⁺, αβ TCR⁺ T cells. It was found that approximately 32% of both CD4 and CD8 T cell clones that were breast carcinoma-reactive, were actually responding to MHC-presented OFA/iLRP peptides (8). This means that many T cells have been activated in breast carcinoma patients that can recognize non OFA/iLRP tumor-expressed antigens, but a significant number of anti-tumor T cells in breast carcinoma patients are specific for OFA/iLRP epitopes. It also means that anti-tumor T cells which are induced during cancer development in these patients are typical CD3⁺, αβ TCR⁺ T cells and their tumor antigen recognition MHC-restricted. Although some have found it difficult to identify MHC-restricted anti-breast cancer T cells from cancer patients (93, 94), our work (8) (described in this section) shows that typical CD4 and CD8 anti-OFA/iLRP T cells are induced during breast carcinoma development. Thus, unusual stimulation techniques for utilizing these anti-tumor T cells in immunotherapy should not be required.

All of the CD4, OFA/iLRP-specific T cell clones established from the breast cancer patients' peripheral blood were Th1 cells which secreted γ-interferon, but not IL-4 or IL-10 upon stimulation with irradiated, autologous breast carcinoma cells in the presence of irradiated, autologous T cell-depleted peripheral blood mononuclear cells (PBMC) acting as antigen-presenting cells plus IL-2. The CD8, OFA/iLRP-specific T cell clones established from

the breast cancer patient's peripheral blood were split in their cytokine profiles. Some of the CD8 clones secreted γ-interferon, but not IL-4 or IL-10 upon stimulation with irradiated, autologous breast carcinoma cells in the presence of irradiated, autologous T cell-depleted peripheral blood mononuclear cells (PBMC) acting as antigen-presenting cells plus IL-2. These clones were also cytotoxic to autologous breast carcinoma cells and their cytotoxicity could be blocked by anti-class I MHC monoclonal antibody showing once again that the effector T cells showed typical MHC restriction in recognition of tumor-expressed OFA/iLRP. The CD8, OFA/iLRP-specific T cell clones that were not able to kill autologous breast carcinoma cells in a typical cytotoxicity assay, did not secrete either γ-interferon or IL-4. They, however, did secrete IL-10. We previously showed in mouse studies that IL-10 can inhibit cytotoxic T cell killing (9). Indeed, in these studies, if a neutralizing monoclonal anti-IL-10 antibody was included in the media used in the cytotoxicity assay, the non-cytotoxic CD8 T cell clones became cytotoxic to autologous breast carcinoma cells, but that cytotoxicity is blocked by a monoclonal anti-class I MHC antibody. So, even these CD8 T cells are typical MHC-restricted T cells. It is unclear why some IL-10-secreting, OFA/iLRP-specific T cells are induced. It could be due to tumor products secreted to try and weaken anti-tumor effector immunity. It could also be an immune T cell response mounted to try and inhibit tumor cell metastasis due to the fact that OFA/iLRP is involved in activating the tumor-secreted metalloproteinases involved in metastasis (95) and IL-10 induces tissue inhibitor of those metalloproteinases (96).

Because the IL-10-secreting, CD8, OFA/iLRP-specific T cell clones can inhibit CD8 anti-tumor cytotoxic T cell activity through IL-10, it may be that a breast carcinoma patient who develops too many IL-10-secreting, CD8, OFA/iLRP-specific T cells or develops them at the wrong part of the anti-tumor immune response could have a worse prognosis than a patient with fewer IL-10-secreting, CD8, OFA/iLRP-specific T cells. This view was further strengthened by data from this breast carcinoma study (8). The two patients who had the highest number of clonable CD8, OFA/iLRP-

specific, IL-10-secreting T cells both had tumor recurrences after undergoing the normal surgery and chemotherapy treatments. However, the other patients, who had significantly fewer clonable IL-10-secreting, CD8, OFA/iLRP-specific T cells had successful outcomes after their normal anti-breast carcinoma surgery and chemotherapy treatments. While this study had too small a number of patients from which to prove the general prognostic use of enumerating IL-10-secreting, CD8, OFA/iLRP-specific patient T cells, it is of interest that only those with the highest number of IL-10-secreting clones had tumor recurrence. This may mean that immunization with OFA/iLRP in a mode that induces only or, at least, primarily Th1 and cytotoxic CD8 T cells could be used in immunotherapy. One such mode would be by immunizing with autologous dendritic cells pulsed with OFA/iLRP or OFA/iLRP peptides that induce Th1 and cytotoxic T cells, but not IL-10-secreting CD8 T cells.

2. Renal Cell Carcinoma Patients:

OFA/iLRP is expressed in all renal carcinoma samples tested (n=13) using flow cytometry and an OFA-specific monoclonal antibody. Peripheral blood mononuclear cells obtained from patients with metastatic renal cell carcinoma responded to stimulation with recombinant OFA/iLRP by proliferating and producing the cytokines interferon- γ and IL-10. OFA/iLRP specific immunity in patients with metastatic renal cell carcinoma was stimulated by vaccination with tumor lysate pulsed dendritic cells. This specific immunity to OFA/iLRP was associated with enhanced levels of IFN- γ and substantial regression of all metastases after the second vaccination of the patient. Interestingly, relatively low levels of OFA/iLRP on presenting dendritic cells prevented tolerization of OFA/iLRP specific T cells. In contrast high level expression of OFA/iLRP in tumors may lead to cross presentation of OFA/iLRP to CD8⁺ T cells, which may result in the development of OFA/iLRP specific regulatory T cells that produce IL-10 (97).

In 27 evaluable patients with metastatic renal cell carcinoma receiving vaccinations with CD83⁺ autologous monocyte-derived dendritic cells loaded with lysate of cultured autologous or allogeneic permanent tumor cells (A-498) as well as KLH as control and helper

antigen, 2 patients did not show any evidence of disease (complete response). One patient had an objective partial response. Seven patients had stable disease, the remaining 17 patients had progressive disease. In 5 of 6 patients tested, enhanced immune responses against OFA/iLRP could be detected. The strongest responses against OFA/iLRP were detectable in 2 patients with complete response and partial response, respectively (98). In clinical studies in progress, a majority of patients with advanced renal carcinoma appear to be giving a potent objective response to autologous DC cells loaded *in vitro* with purified OFA (personal communication).

3. Patients with hematologic malignancies:

OFA/iLRP is strongly expressed in all hematologic malignancies but not on cell subpopulations purified from peripheral blood of patients with AML and of healthy donors like CD19⁺ (B lymphocytes), CD3⁺CD8⁺ (T lymphocytes), CD14⁺ (monocytes) cells, CD34⁺ peripheral blood progenitor cells, and immature and matured dendritic cells (99).

Recently, we investigated the capability of OFA/iLRP-specific cytotoxic T lymphocyte to kill OFA/iLRP-expressing hematologic targets. CTLs were generated from healthy HLA-A*0201-positive volunteers by incubating T cells with autologous dendritic cells transfected with OFA/iLRP RNA. OFA/iLRP specific CTL lysed HLA-A2⁺ OFA/iLRP⁺ tumor cells, including several lymphoma and Leukemia cell lines, as well as fresh leukemic targets from patients with acute myeloid leukemia (AML) and chronic lymphatic leukemia (CLL), indicating that OFA/iLRP-derived peptides are naturally processed and presented by hematologic tumors. Healthy OFA/iLRP-negative target cells (CD14⁺ monocytes, activated B cells, Dcs, bone marrow cells) were not attacked by OFA/iLRP-specific CTLs (99). Furthermore, in an established murine B-cell lymphoma model (A20), treatment with syngeneic Dcs transfected with OFA/iLRP-coding RNA resulted in powerful antitumor effects in a significant portion of mice (99).

Polyclonal OFA/iLRP-specific CTL generated with autologous OFA/iLRP RNA-transfected dendritic cells contain T lymphocytes capable of recognizing 2 OFA/iLRP-derived peptides,

selected according to the motif for HLA-A*0201, as demonstrated by their potential to kill T2 cells loaded with these peptides. This recognition suggests that at least 2 clonotypes had been generated by using autologous OFA/iLRP RNA-transfected Dcs as stimulator cells (99).

These data show that OFA/iLRP can be used as a target for T-cell-based immunotherapeutic strategies against three different types of malignancies

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