

## Commentary

# Biomarker vaccines

Irun R. Cohen

Department of Immunology; The Weizmann Institute of Science; Rehovot, Israel

**Key words:** biomarker, vaccine, HSP60, conjugate vaccine, immunomodulation

## Biomarkers Simplify Complexity and Make Possible Healthcare

A biomarker is a diagnostic indicator of a medical condition—a sign that provides a physician or healthcare specialist with the critical information needed to diagnose, monitor, prognosticate and/or treat a disease or other condition of biological or medical interest.<sup>1</sup> Organisms and their maladies are exceedingly complex, but biomarkers, by representing faithfully the underlying reality, allow the physician to reduce the complexity of disease and thereby deal with it. A biomarker is a small bit of information that stands in for the enormous and unattainable facts of biological existence. Fever, for example, serves and has served patients, shamans, and other healthcare practitioners as a useful biomarker for thousands of years before Western medical science discovered the immune system, pathogens, cytokines and all the rest; fever is a faithful biomarker because it is a simple, measurable outcome of the enormous complexity of known and unknown factors that generate it. Healthcare would just not be possible without biomarkers.

## The Bottom Line

Here, I submit that:

- (1) the immune system is a healthcare system;
- (2) the immune system, like any good doctor or shaman, uses biomarkers to manage the body;
- (3) pathogens use biomarkers to subvert the immune system to their own ends; and
- (4) effective vaccines can be constructed using biomarkers chosen for desirable ends; I bring the example of HSP60 peptides in conjugate vaccines.

## The Immune System is a Healthcare System that Manages Inflammation

Inflammation is a physiological process set into motion by injury and that leads to healing.<sup>2</sup> Processes of inflammation that

are dynamically tuned to the particular disease and its resolution are required to maintain the organism, at least for a while, in the face of the wear, tear, parasites, transformations, and accumulating entropy that threaten life.<sup>3</sup> Inflammation carries a bad name because dysregulated inflammation can cause disease; but on the whole, we and the other multi-cellular creatures owe our survival to well-disposed inflammation. Inflammation maintains health, so whoever controls inflammation is by definition a provider of healthcare. Now the immune system, adaptive and/or innate, controls inflammation;<sup>3,4</sup> ergo, the immune system is a healthcare system.

## The Immune System Uses Biomarkers

The classical clonal selection theory of adaptive immunity, as enunciated by Burnet<sup>5</sup> and others,<sup>6</sup> proposed that the immune response was wholly determined by the foreign antigens that happened to enter the body and activate specific receptor-bearing lymphocytes. We now know, however, that antigens do not, by themselves, determine the biologic output of an immune response; the biologic meaning of an immune response results from the type of inflammation generated by the responding cells and molecules, a response both of the immune system and of the body.<sup>3,4</sup> The immune system can respond to a given antigen with a variety of effector, suppressor, and regulatory cells and molecules—the dynamic mix of these outputs orchestrates the inflammatory response to infection, injury, infarction, wounds, neoplasia and other insults to health. These biologic outputs depend in large part on the innate adjuvant signals that accompany the antigen.<sup>7</sup> Thus, adjuvant signals, which interact with innate receptors, are key actors in determining the biologic meaning of any immune response.<sup>3</sup> Adjuvant signals that determine the biologic meaning of an immune response can be envisioned as immune biomarkers—marker molecules that interact with innate immune receptors and thereby influence the nature of the ensuing inflammatory response.

As I shall discuss in greater detail below, heat shock protein (HSP) molecules are telling examples of immune biomarkers: Upregulation of HSP molecules (HSP60, HSP70, HSP90 and others) are infallible signs that cells are under stress and need immune attention—whatever the cause; HSP molecules are biomarkers to the immune system as is fever (and other signs) to the doctor.

Correspondence to: Irun R. Cohen; Department of Immunology; The Weizmann Institute of Science; Rehovot 76100 Israel; Email: irun.cohen@weizmann.ac.il

Submitted: 05/22/09; Accepted: 06/17/09

Previously published online as a *Human Vaccines* E-publication:  
<http://www.landesbioscience.com/journals/vaccines/article/9266>

Note that the biology of the immune response integrates individual immune experience with the immune experience of the species.<sup>3</sup> Antigen receptors are not inherited in the germline but arise anew somatically in the individual; thus, one's repertoire of antigen receptors records one's personal immune experience. Innate receptors, in contrast, are inherited in the germline; thus, one's repertoire of innate immune receptors records the immune evolution of one's species.

### Pathogens Use Biomarkers to Subvert Immune Surveillance

In the beginning, vaccination was held to be a simple matter: just immunize the subject to an antigen or antigens of the pathogen; any immune response to a foreign antigen will trigger an immune response that rids the body of the pathogen.<sup>8</sup> We now know that effective vaccination is not so simple: on the one hand, the immune repertoire is complex; there are a great many variant types of immune molecules and inflammatory responses deployed by the immune system; on the other hand, many pathogenic viruses and bacteria have co-evolved with the immune system of the parasitized species and have evolved to trigger types of immune response that work to their own advantage. Think of the immune response variants that lead to life-long parasitism and parasite dissemination despite host "immunity"—witness cytomegalovirus, *M. tuberculosis*, HIV, *T. pallidum*, and the like. Such infectious pathogens selectively activate choice components of the immune repertoire. Even tumor cells evolve—at the scale of the individual—and are selected, as a consequence of the host-tumor interaction, to manage the host immune response.<sup>9</sup> These pathogens deploy cytokines and other innate cytokine-like adjuvant signals and biomarkers to establish a protected niche in the body.<sup>10</sup>

### Biomarker Vaccines

An effective vaccine will activate the subject's immune system in a way that prevents a future disease (a preventive vaccine) or that arrests a disease already underway (a therapeutic vaccine). My point here is that an effective vaccine should focus on the pathophysiology of the host-parasite interaction, and not on the pathogen alone; a naïve, unsophisticated immune response to the pathogen—in the case of HIV, or a tumor, or a Group A Streptococcus, for example—might just be exploited by the pathogen to further the infection<sup>10,11</sup> or by the tumor to enhance its growth,<sup>9</sup> or might lead to an autoimmune disease.<sup>13</sup> The effective vaccine should be fashioned to provide the immune system with the information it needs to prepare an immune response that annuls or circumvents the pathogen's game plan. An effective vaccine must be tuned to the specific host-parasite interaction and, most importantly, the vaccine must induce an immune response that favors the host in its inflammatory dialog with the pathogen. An effective vaccine can engineer the host immune response by using effective biomarkers.

### HSP60 Conjugate Vaccines

HSP60 functions as a chaperone inside the cell, but outside the cell, self-HSP60 functions as a biomarker molecule to the immune system.<sup>7</sup> Since HSP60 is upregulated by any form of stress, HSP60

is a reliable biomarker signal of serious trouble: infection, trauma, metabolic insult, genomic aberration, and other factors that might require immune intervention. Because of its importance as a reliable biomarker, HSP60 is recognized by a collective of different immune receptors: HSP60 is a natural antigen for T cells<sup>13</sup> and B cells<sup>14</sup> and an innate ligand for TLR4 or TLR2 on T cells,<sup>15</sup> B cells,<sup>16</sup> macrophages and dendritic cells.<sup>17</sup> HSP60 seems to act as an internal adjuvant that can both upregulate and downregulate the nature and strength of immune inflammation depending on the particular HSP60 epitope, the concentration of HSP60 molecules and the responding immune cell.<sup>7</sup> HSP60 is thus an important biomarker component in the immunological homunculus,<sup>18,19</sup> both as a natural self-antigen and as a self-ligand for innate receptors.<sup>20</sup>

For these reasons, I and my colleagues have developed conjugate vaccines based on combining a specific pathogen epitope with a peptide epitope of the mammalian HSP60 molecule. We have used a peptide of self-HSP60, termed p458, to formulate subunit vaccines by conjugating p458 to various pathogen virulence molecules such as the capsular polysaccharides (CPS) of Salmonella,<sup>21</sup> Pneumococcus<sup>22</sup> or Meningococcus types C and B.<sup>23</sup> Some bacterial HSP60 peptides too are agonists for effective innate receptors, and can also serve in effective conjugate vaccines; the phenomenon is not limited to self.<sup>21</sup> We have also linked HSP60 peptide p458 to peptide epitopes of West Nile Virus (in preparation) and murine CMV.<sup>24</sup> These conjugate vaccines are effective because the p458 component of the conjugate provides T-cell help and the p458-CPS conjugate also activates innate TLR4 signaling in antigen presenting cells.<sup>25</sup> The basic idea is to provide the immune system with a conjugation of information about the pathogen—the target antigen—with a host biomarker—the HSP component. The host biomarker moiety flags the antigen and so induces the host response into an effectively protective mode (Fig. 1). Note that these conjugate vaccines are effective without the need for added adjuvant,<sup>21-23</sup> a factor that adds to safety. The detailed results can be seen in the publications. The lessons relevant to our present discussion can be summarized thusly:

- (1) The self-HSP60 peptide conjugate can be more effective in generating T-dependent antibodies and memory than foreign carrier molecules and can increase resistance to lethal challenge by a million fold—exemplified in a Pneumococcal vaccine;<sup>22</sup>
- (2) The self-HSP60 peptide conjugate can convert non-immunogenic or very poorly immunogenic pathogen molecules into strong immunogens—exemplified in a vaccine to Meningococcus B;<sup>23</sup>
- (3) The self-HSP60 peptide conjugate can induce immune memory and cytotoxic T cells that abrogate the persistence of a pathogen in a naturally protected site—exemplified by the eradication of murine CMV from its hideout in the salivary glands.<sup>24</sup> Immunization with the virus itself could not induce the mouse to clear CMV from its salivary glands; the conjugate virus-host biomarker vaccine was successful.

We are presently developing a prototype anti-tumor vaccine by conjugating a tumor-associated antigen to an HSP epitope; the aim is to amplify immune surveillance by presenting the tumor antigen in the context of a biomarker that will serve the host immune

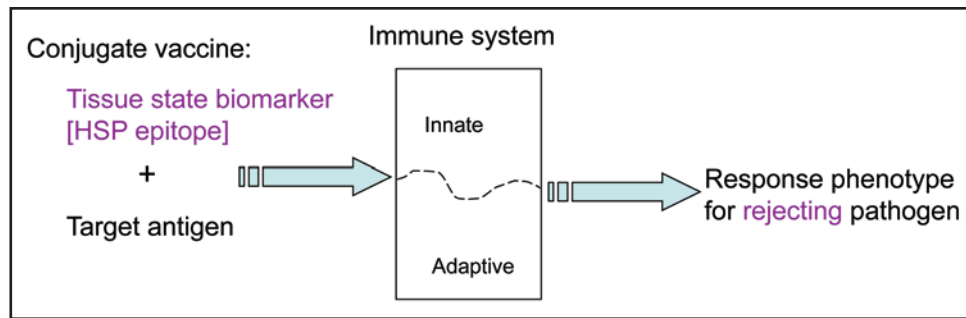


Figure 1. Biomarker conjugate vaccines. A vaccine antigen alone does not specify the biological phenotype of the immune response elicited by that antigen; the type of effector or suppressive response to the antigen is determined by adjuvant biomarkers that can activate a variety of innate immune receptors. Infectious agents and tumor cells often provide adjuvant biomarkers that divert the immune response to their own advantage. However, an effective vaccine can be engineered by conjugating to the target antigen a biomarker molecule, such as an HSP epitope, that will elicit the type of response needed to reject the pathogen.

response. We are also testing whether selected peptides derived from the HSP60 sequences of bacteria (non-self) can also be used to prepare conjugate vaccines with the same properties as the self-HSP60 peptide conjugates;<sup>21</sup> such peptides might circumvent the need to vaccinate with self-peptides. But self-HSP60 peptides appear to be safe in humans; we are even born with autoantibodies to HSP60,<sup>14</sup> and T cells reactive to HSP60.<sup>26</sup> The capacity to recognize HSP60 from birth is certainly compatible with HSP60 as an immune biomarker<sup>20</sup> and with its safety. Indeed, a different self-HSP60 peptide is currently in phase 3 clinical trials to treat type 1 diabetes mellitus.<sup>27</sup> The message is that the immune system is an accomplished healthcare specialist; vaccines should be designed to allow us to talk to it in its own biomarker language.<sup>3</sup>

## References

- Cohen IR. Biomarkers, self-antigens and the immunological homunculus. *J Autoimmun* 2007; 29:246-9.
- Ebert RH. The Inflammatory Process. In *General Pathology* Zweifach BW, Grant L, McCluskey RT, eds. Academic Press, New York, p1 1965.
- Cohen IR. *Tending Adam's Garden: Evolving the Cognitive Immune Self*. Academic Press, London UK 2000.
- Cohen IR. Discrimination and dialogue in the immune system. *Semin Immunol* 2000; 12:215-9.
- Burnet M. *Auto-immunity and Auto-immune Disease*. Philadelphia: Davis FA Company 1972.
- Langman RE, Cohn M. A minimal model for the self-nonsel discrimination: a return to the basics. *Semin Immunol* 2000; 12:189-95.
- Quintana FJ, Cohen IR. Heat shock proteins as endogenous adjuvants in sterile and septic inflammation. *J Immunol* 2005; 175:2777-82.
- Cohn M, Langman RE. To be or Not to be riddled?—That is the question addressed by the associative antigen recognition model. *Scand J Immunol* 2002; 55:318-23.
- Gorter A, Meri S. Immune evasion of tumor cells using membrane-bound complement regulatory proteins. *Immunol Today* 1999; 20:576-82.
- Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol* 2005; 3:948-58.
- Collins KL, Baltimore D. HIV's evasion of the cellular immune response. *Immunological Reviews* 2006; 168:65-74.
- Stollerman GS. Rheumatic fever. *AMA Arch Intern Med* 1956; 98:211-20.
- Abulafia-Lapid R, Elias D, Raz I, Keren-Zur Y, Atlan H, Cohen IR. T Cell Proliferative Responses of Type 1 Diabetes Patients and Healthy Individuals to Human hsp60 and its Peptides. *J Autoimmun* 1999; 12:121-9.
- Merbl Y, Zucker-Toledano M, Quintana FJ, Cohen IR. Newborn humans manifest autoantibodies to defined self-molecules detected by antigen microarray informatics. *J Clin Invest* 2007; 117:712-8.
- Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR. Heat shock protein 60 enhances CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cell function via innate TLR2 signaling. *J Clin Invest* 2006; 116:2022-32.
- Cohen-Sfady M, Nussbaum G, Pevsner-Fischer M, Mor F, Carmi P, Zanin-Zhorov A, et al. Heat Shock Protein 60 Activates B Cells via the TLR4-MyD88 Pathway. *J Immunol* 2005; 175:3594-602.
- Munz C, Steinman RM, Fujii S. Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J Exp Med* 2005; 202:203-7.
- Cohen IR, Young DB. Autoimmunity, microbial immunity and the immunological homunculus. *Immunol Today* 1991; 12:105-10.
- Cohen IR. The cognitive paradigm and the immunological homunculus. *Immunol Today* 1992; 13:490-4.
- Cohen IR. Real and artificial immune systems: computing the state of the body. *Nature Rev Immunol* 2007; 7:569-74.
- Konen-Waisman S, Fridkin M, Cohen IR. Self and foreign 60-kilodalton heat shock protein T cell epitope peptides serve as immunogenic carriers for a T cell-independent sugar antigen. *J Immunol* 1995; 154:5977-85.
- Amir-Kroll H, Nussbaum G, Cohen IR. Proteins and their derived peptides as carriers in a conjugate vaccine for streptococcus pneumoniae: Self-heat shock protein 60 and tetanus toxoid. *J Immunol* 2003; 170:6165-71.
- Amir-Kroll H, Riveron L, Sarmiento ME, Sierra G, Acosta A, Cohen IR. A conjugate vaccine composed of a heat shock protein 60 T-cell epitope peptide (p458) and *Neisseria meningitidis* type B capsular polysaccharide. *Vaccine* 2006; 24:6555-63.
- Rouvio O, Dvorkin T, Amir-Kroll H, Atias D, Cohen IR, Rager-Zisman B, Porgador A. Self HSP60 peptide serves as an immunogenic carrier for a CTL epitope against persistence of murine cytomegalovirus in the salivary gland. *Vaccine* 2005; 23:3508-18.
- Cohen N, Stolarsky-Bennun M, Amir-Kroll H, Margalit R, Nussbaum G, Cohen-Sfady M, et al. Pneumococcal capsular polysaccharide is immunogenic when present on the surface of macrophages and dendritic cells: TLR4 signaling induced by a conjugate vaccine or by lipopolysaccharide is conducive. *J Immunol* 2008; 180:2409-18.
- Munk ME, Schoel B, Modrow S, Karr RW, Young RA, Kaufmann SH. T lymphocytes from healthy individuals with specificity to self-epitopes shared by the mycobacterial and human 65-kilodalton heat shock protein. *J Immunol* 1989; 143:2844-9.
- Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* 2001; 358:1749-53.