Network cross-reactivity: DNA vaccination with HSP70 or HSP90 modulates immunity to HSP60 and inhibits adjuvant arthritis

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Summary

Adjuvant arthritis (AA) is induced using *Mycobacterium tuberculosis* (Mt). Immunization with mycobacterial heat shock proteins (HSPs) inhibits AA, supposedly by the induction of T cell responses cross-reactive with self-HSPs. Indeed, vaccination with the human 60-kDa HSP (HSP60) inhibits AA. In this work we studied the control of AA by self-HSP molecules other than HSP60 using DNA vaccines coding for the human 70 kDa (pHSP70) or 90 kDa (pHSP90) HSPs. pHSP70 and pHSP90 down-regulated the arthritogenic T-cell response and inhibited AA. In addition, both pHSP70 and pHSP90 induced T-cell responses to HSP60, and vaccination with pHSP70 triggered the release of endogenous HSP60 to the circulation. Thus the regulatory activities of HSP on inflammation might involve two types of cross-reactivity: *molecular cross-reactivity* exists between microbial and self-HSPs, and *network cross-reactivity* exists between different self-HSPs.

Introduction

AA is induced in Lewis rats by immunization with Mt (*1*). T-cells reactive with the mycobacterial 65-kDa HSP (HSP65) mediate both the pathogenesis of AA (*2*), and its regulation, probably due to cross-reactivity with the mammalian 60-kDa HSP (HSP60) (*3*). Indeed, vaccination with fragments (*4*) or whole human HSP60 (*5*) inhibits AA. However, although vaccination with the mycobacterial 10 kDa- or the 71-kDa (HSP71) HSPs also inhibits AA (*6, 7*), little is known about the regulatory role of T cells reactive with self-HSP molecules other than HSP60 in AA.

Here we analyzed the role of T-cell responses to the mammalian HSP70 and HSP90 in AA using DNA vaccines. DNA Vaccination with pHSP70 or pHSP90 inhibited the arthritogenic response, controlled AA, and surprisingly, also induced a cross-reactive immune response to HSP60.

Materials and Methods

The Mt176-190 peptide of HSP65, and recombinant HSP60 and HSP90, were prepared as described (8). HSP65 was given by Prof. Ruurd van der Zee (Faculty of Veterinary Medicine, Utrecht, The Netherlands); tuberculin purified protein derivative (PPD) and HSP71 were provided by the Statens Seruminstitut (Denmark); human HSP70 and ovoalbumin (OVA) were purchased from Sigma (Israel); and Mt Strain H37Ra and incomplete Freund's adjuvant (IFA) were purchased from Difco (USA).

The full-length cDNAs of the human hsp70 or hsp90α (accession numbers M11717 and NM_005348, respectively) were cloned into pcDNA3 (Invitrogen, The Netherlands), and checked to be functional in vitro and in vivo (data not shown). AA was induced and scored in DNA vaccinated or control rats as described (8). T-cell proliferation and cytokine secretion assays were performed 26 days after the induction of AA as described (8). Serum HSP60 was quantified in serum as reported (8).

Results

We investigated the effects on AA of DNA vaccination with pHSP70 or pHSP90. The empty control vector pcDNA3 did not inhibit the development of AA, however vaccination with pHSP70 or pHSP90 induced a significantly milder arthritis (Figure 1).

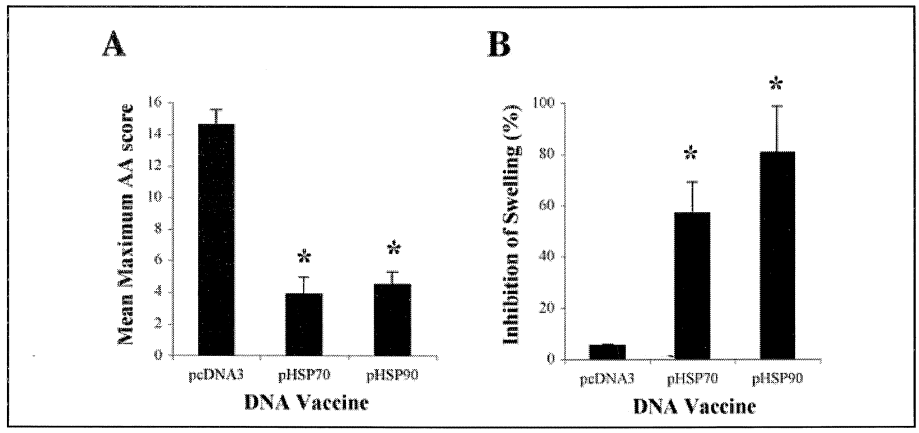

*Figure 1: Vaccination with pHSP70 or pHSP90 inhibits AA. A. Maximal mean AA score (± SEM) B. Inhibition of leg swelling (Mean ± SEM) relative to an untreated group. * $p < 0.005$ compared to pcDNA3-vaccinated rats.*

Table I: Immune response in DNA-vaccinated rats

T-cell Response	Antigen	pcDNA3	pHSP70	pHSP90
Proliferation	PPD	4 ± 1.1	12.6 ± 4.5 *	8.7 ± 4.1 *
	HSP71	1.7 ± 1.1	7.3 ± 2.4 *	8.3 ± 3.2 *
	HSP65	2 ± 0.6	3.5 ± 1.4 *	3.8 ± 1 *
	Mt176-90	2.2 ± 0.7	3.6 ± 0.4 *	3.4 ± 0.7 *
IFNγ	PPD	1547 ± 234	748 ± 84 *	357 ± 45 *
	HSP71	840 ± 148	685 ± 105	540 ± 60 *
	HSP65	987 ± 167	475 ± 83 *	365 ± 68 *
	Mt176-90	2145 ± 345	(-)	(-)
IL-10	PPD	193 ± 34	415 ± 58 *	950 ± 176 *
	HSP71	73 ± 14	268 ± 45 *	345 ± 48 *
	HSP65	(-)	115 ± 23 *	45 ± 7 *
	Mt176-90	(-)	(-)	(-)
TGFβ1	PPD	(-)	1050 ± 258 *	530 ± 132 *
	HSP71	(-)	(-)	421 ± 82 *
	HSP65	(-)	(-)	4800 ± 870 *
	Mt176-90	(-)	(-)	5570 ± 1025 *
Proliferation	HSP60	1.2 ± 0.1	3.8 ± 1 *	2.3 ± 0.3
	HSP70	1.8 ± 0.2	6.9 ± 0.6 *	ND
	HSP90	2.2 ± 0.4	ND	4.8 ± 0.9 *
IFNγ	HSP60	(-)	198 ± 45 *	(-)
	HSP70	(-)	(-)	ND
	HSP90	(-)	ND	183 ± 38 *
IL-10	HSP60	(-)	55 ± 15 *	(-)
	HSP70	(-)	322 ± 56 *	ND
	HSP90	30 ± 15	ND	120 ± 24 *
TGFβ1	HSP60	20 ± 10	935 ± 315 *	20 ± 10 *
	HSP70	20 ± 10	20 ± 10	ND
	HSP90	(-)	ND	105 ± 31 *

Proliferation data are shown as the mean stimulation index ± SEM.
Cytokine secretion is shown as the mean pg/ml ± SEM.
(-), below detection (<15 pg/ml) ND, not determined; * $p<0.05$ compared to pcDNA3.

The progression of AA is driven by Th1 cells reactive with mycobacterial antigens (*1, 4, 8*). In contrast, the inhibition of AA usually leads to increased proliferation, and a Th1 to Th2/3-like shift in the Mt-specific immune response (*4, 8, 9*). Table I shows that the rats protected by pHSP70 or pHSP90 vaccination showed stronger proliferative responses than did the control rats to Mt-derived antigens. Moreover, LNC from pHSP70- or pHSP90-treated rats secreted significantly less IFNγ and more IL-10 than control rats upon stimulation with the same antigens. LNC of pHSP70-vaccinated rats only secreted TGFβ1 upon activation with PPD, while LNC of the pHSP90 group secreted TGFβ1 upon stimulation with the four Mt antigens used in this study. None of

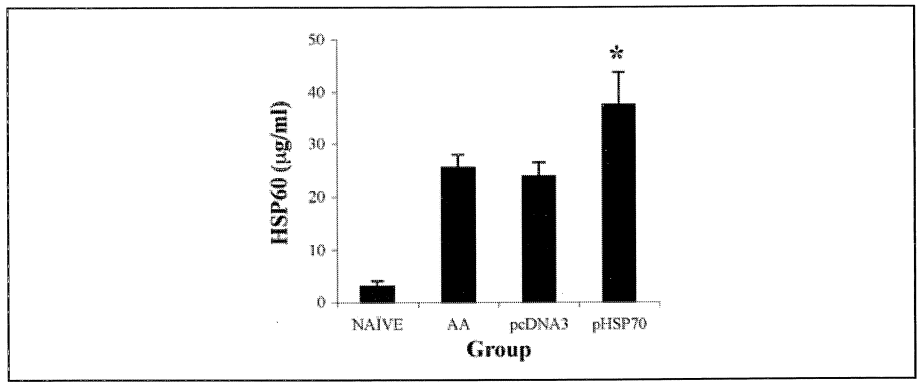

*Figure 2: pHSP70 vaccination up-regulates serum HSP60. Mean HSP60 levels (± SEM) in serum. *p < 0.05 compared with the pcDNA3 group.*

the experimental groups showed significant T-cell responses to GST, and they did not differ in their responses to Con A (data not shown). Thus, vaccination with pHSP70 or pHSP90 leads to a Th1 to Th2/3-like in the arthritogenic response and the inhibition of AA.

We also studied the effect of vaccination with pHSP70 or pHSP90 on the T-cell responses to HSP70, HSP90 and HSP60 (Table I), since DNA vaccination with HSP60 activates HSP60-specific T cells and inhibits AA (*4, 8*). pHSP70 and pHSP90 induced antigen-specific proliferative responses to HSP70 and pHSP90, respectively. The HSP90-specific T cells secreted IFNγ, IL-10 and TGFb1 upon stimulation with HSP90, and the HSP70-reactive T cells secreted IL-10 upon stimulation with HSP70, but not IFNγ or TGFβ1. Unexpectedly, DNA-vaccination with pHSP70 or pHSP90 induced significant T-cell proliferation to HSP60, accompanied by the secretion IFNγ, TGFβ1 and IL-10 detected in LNC taken from pHSP70-vaccinated rats. Thus, vaccination with pHSP90 or pHSP70 can activate HSP60-specific T-cell immunity.

Serum HSP60 has been linked to inflammation, therefore we studied whether the induction of AA and/or vaccination with pHSP70 might increase HSP60 levels in serum. Figure 2 shows that AA itself increased serum HSP60, and the HSP60 levels were further increased by vaccination with pHSP70.

Discussion

In this work we studied the effects of DNA vaccination with HSP90 or HSP70 on AA. Vaccination with pHSP70 or pHSP90 led to a Th1 to Th2/3 shift in the response to Mt-derived antigens, associated with inhibition of AA. T-cell responses to Mt, and in particular to Mt176-

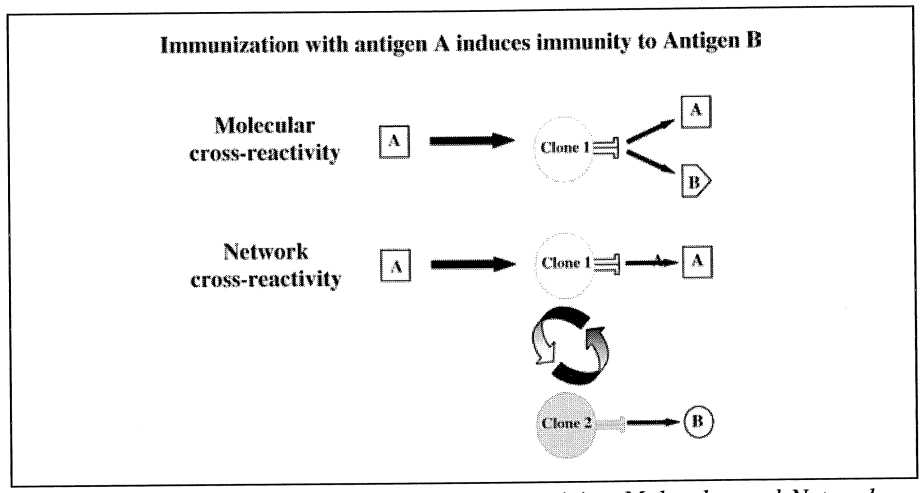

Figure 3: Two types of immunological cross-reactivity: Molecular and Network.

190 seem to drive the arthritogenic response. Indeed, a T-cell clone specific for the 176-190 region of HSP65 cross-reacts with collagen and can adoptively transfer AA (*1, 10*). Thus, the pHSP70 and pHSP90 vaccines might inhibit AA by modulating the T-cell response to Mt, and in particular to Mt176-90.

DNA vaccination with HSP60 (*8*) and vaccination with HSP60 peptides (*4*) shift the Mt-specific T-cell response towards Th2/3 and inhibit AA. In human rheumatoid arthritis (*11*) and juvenile chronic arthritis (*12*), an HSP60-specific T-cell response is associated with a better prognosis and milder symptoms. Vaccination with mycobacterial HSP71 (*6, 9, 13*) or some of its T-cell epitopes (*9, 14*) inhibits AA and induces regulatory T cells cross-reactive with mammalian HSP70. BiP, a member of the HSP70 family of proteins which has 64 % of homology with the HSP70 used in our studies (accession number M11717), also inhibits AA (*15*). Although they differ in their cellular localization, both BiP and HSP70 are stress-inducible proteins. Therefore, it should be tested whether the immuno-modulatory activity of HSP70 relies on its induction by stress. Nevertheless, taken together the results presented herein suggest a regulatory role in human and experimental arthritis for HSP-specific T cells.

Surprisingly, immunization with pHSP70 or pHSP90 induced a T-cell response to HSP60, even though HSP60, HSP70 and HSP90 share no sequence homology (data not shown). One possible explanation for this observation is self-vaccination with endogenous HSP60 triggered by the pHSP70 and pHSP90 DNA vaccines. This hypothesis is sup-

ported by the detection of increased levels of circulating HSP60 in pHSP70-vaccinated rats (Figure 2). Since proteins encoded by DNA vaccines are detectable in the blood after vaccination (*16*) and HSPs are ligands for innate receptors (*17*), it is conceivable that HSP60, HSP70 and HSP90 mutually up-regulate their expression via an innate receptor-mediated mechanism. This and other possible explanations for HSP immune cross-talk need further investigation.

Does cross-reactivity between HSP70 or HSP90 and HSP60 play a role in regulating AA? A detailed study of the T cell response to HSP60 induced by pHSP70 revealed that several HSP60 epitopes recognized by pHSP70-vaccinated rats were previously identified as regulatory in AA (data not shown). Thus the regulatory properties of HSP70 and HSP90 in AA might be reinforced by the induction of an immune response directed to regulatory HSP60 epitopes.

Note that the textbook definition of immunological cross-reactivity cannot account for the HSP60/HSP70 cross-reactivity seen here. For this reason, we propose a second definition for cross-reactivity. We define *molecular cross-reactivity* as the classical cross-reactivity that exists between antigens that share sequence homology and are therefore recognized by the same T or B cell clones (Figure 3). We define *network cross-reactivity* as the immune connection existing between molecules that bear no sequence homology, like HSP60 and HSP70, but whose specific immune responses are interconnected (by self-vaccination or any other mechanism). Thus, *network cross-reactivity* is a consequence of the organization of the immune network and does not rely on the existence of single T or B cell clones that recognize both antigens (Figure 3). The regulatory properties of HSPs might therefore result both from the *molecular cross-reactivity* existing between self and microbial HSPs, and from the *network cross-reactivity* connecting different endogenous HSPs.

The study of the anti-inflammatory mechanisms mediated by HSP could lead to the design of novel therapies for autoimmunity - therapies to reinforce HSP-based built-in physiological mechanisms of control of the immune function (*18*). The initial success of the HSP60 peptide p277 in treating human T1DM shows the feasibility of this approach (*19*). Controlled auto-reactivity is needed for the proper functioning of the immune system and body homeostasis (*18*), but might also be exploited for the design of new therapies for autoimmune disease.

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