We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

5,500
Open access books available

135,000
International authors and editors

165M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

12

The Inflammatory Infiltrate in Calcific Aortic Stenosis is Characterized by Clonal Expansions of T Cells and is Associated with Elevated Proportions of Circulating Activated and Effector Memory CD8 T Cells

Robert Winchester and Susheel Kodali
Columbia University, College of Physicians and Surgeons
USA

1. Introduction

For some time, calcific aortic stenosis (CAS) had been considered as a "senile" or "degenerative" response to chronic hemodynamic stress and "wear and tear" on the valve (Pomerance 1972). The concept of inflammation in CAS was etched by Olssen and Otto and their colleagues who were among the first to clearly demonstrate a variable, but often quite appreciable T lymphocyte infiltration in this disease (Olsson, Dalsgaard, et al. 1994, Otto et al. 1994). Further work showed that most T cells infiltrating the valve are concentrated in regions of calcification or arrayed around newly appearing blood vessels that express VEGF, VEGF receptors, ICAM-1 and VCAM-1 (Mazzone et al. 2004, Soini et al. 2003, Wallby et al. 2002). The T cells within the valve tissue exhibit evidence of activation, including expression of CD25 and HLA-DR, while cells in the surrounding valvular mesenchymal tissues also strongly express HLA-DR, consistent with the release of inflammatory mediators such as interferon-γ from activated lymphocytes (Olsson, Dalsgaard, et al. 1994, Olsson, Rosenqvist, et al. 1994). The modified valve mesenchymal cells also express genes characteristic of osteoblasts suggesting that calcification results from an active, regulated osteogenic process (Mazzone et al. 2004, Rajamannan et al. 2003). Since CAS is more prevalent in anatomically variant bileaflet aortic valves and occurs at younger ages in this population, the finding of similar T lymphocyte infiltration in biscupid valves further emphasized the importance of this inflammation in the pathogenesis of CAS (Wallby et al. 2002). However questions regarding the immunologic nature of the T cell infiltrate in the valve, its significance and whether it is part of a broader systemic immune response remain unanswered.
Two principal immune scenarios could account for the conspicuous presence of T cells within CAS valve lesions. One possibility is that the infiltrating T cells consist of large numbers of unexpanded but clonally different lymphocytes that are recruited as a secondary consequence of attractive chemokines released by events such as valvular injury, atherosclerosis or non-antigen specific innate immune system activation of macrophages. This polyclonal T cell infiltration would be analogous to that found in stable atherosclerotic plaques (Li et al. 2005, Oksenberg et al. 1997, Stemme et al. 1991, Swanson et al. 1994). A

second possibility is that the infiltrating T cells are made up by small numbers of highly expanded T cell clones. This would favor the interpretation that some features of an adaptive immune response occur within the valve characterized by selective expansion of certain clones possibly driven by a newly expressed antigen on the valve. This subsequently has the potential for enhanced T cell activation and mediator release associated with clonal expansion resulting in further valve injury.

These two primary scenarios can readily be distinguished by TCR repertoire analysis, which can delineate the clonal composition of the T cell infiltrate in the valve leaflet through structural features of the clonally specific β-chain T cell TCR. This would help determine whether the inflammatory infiltrate in the valve leaflet was primarily polyclonal, suggesting it was a secondary response to injury, or whether the infiltrate contained expanded T cell clones that would imply features of an adaptive immune response occurring within the valve leaflets (Wu et al. 2007).

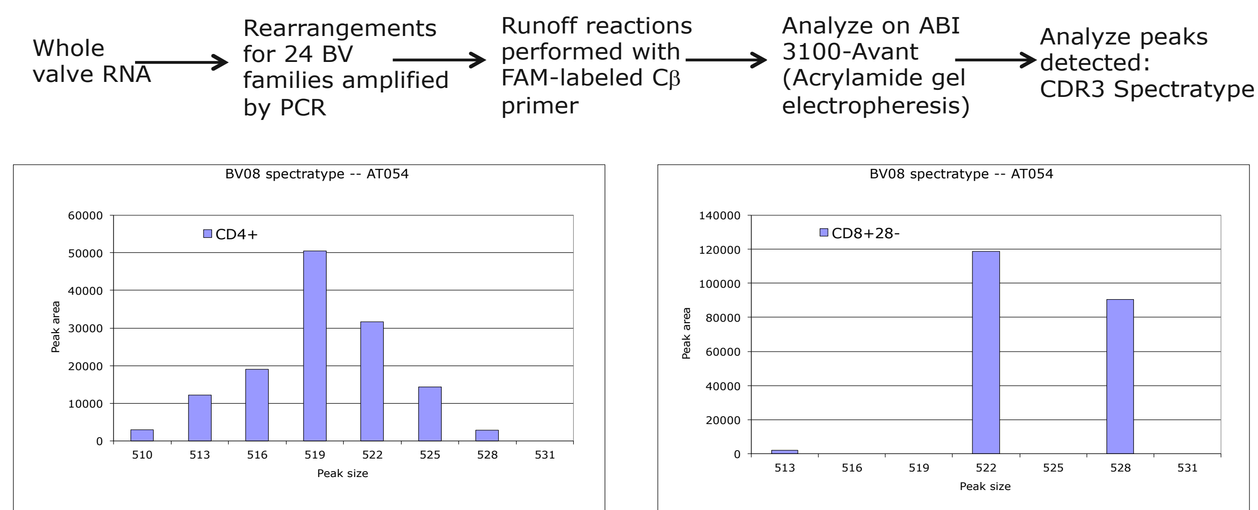
1.1 T cell repertoire analysis background

T cells of either CD8 or CD4 lineage sets are distinguished by different clonally specific T cell receptors (TCRs) that define the T cell's clonotype and determine the clone's specific recognition of the peptide (p)-major histocompatibility complex (MHC). This recognition is the heart of the adaptive immune response. Ligation of a clonal TCR by the appropriate p-MHC complex initiates clonal proliferation of the T cells along with production of cytokines such as γ-interferon. The proliferating clonal progeny all share the same TCR clonotype.

The TCR consists of two chains, designated α and β. Each chain contains a variable and a constant region. In the case of the TCR β-chain, the variable region is composed of three recombined gene elements, the variable (V), diversity (D), and junctional (J) gene segments. The combination of these three segments form a hypervariable region, designated CDR3 that exhibits a clonotypically unique sequence of nucleotides. The amino acids encoded by these nucleotides are responsible for the specificity of the clone's TCR for a particular p-MHC complex.

The clonal proliferation that results from triggering the clone's TCR by this particular p-MHC increases the proportional representation of the responding T cell clones found in that site. The proportional representation of different clones in a T cell repertoire is determined by analysis of the nucleotide structure of the TCR β-chain using PCR analysis. This can be done by sequencing the PCR product and placing identical sequences into groups. The size of each group reflects the extent of clonal expansion and predominance as measured by the frequency distribution of the TCR β-chains from each clone, figure 1. For technical details of this methodology, see (Wu et al. 2007). Since the molecular events underlying the recombination of V, D, and J segments involve exonuclease nibbling from the ends of the joining segments, as well as the incorporation of additional non-germline nucleotides, the overall length of the β-chain is usually randomly altered by some dozens of nucleotides. The distribution of the β-chain lengths, plotted as a histogram, is a technically simpler characterization of the repertoire than detailed sequencing and provides a lower resolution sketch of the composition of an inflammatory repertoire or a given T cell population, figure 1. In this type of length-distribution analysis, sometimes called "spectratyping", a polyclonal repertoire without major clonal expansions exhibits a Gaussian-type of β-chain length distribution, while a repertoire containing a clonal expansion is characterized by a large peak at a particular β-chain length. The form of the plot can be either a histogram

constructed in a spread-sheet program from the experimental results or a direct tracing of the distribution of the fluorescently-labeled PCR product from the machine performing the gel electrophoresis.



The spectratype peak distribution reflects the CDR3 length of the T cell receptor β chains:

-- may be *polyclonal* (normally distributed)
-- may be *oligoclonal* (several peaks, size is not normally distributed): antigen skew is suggested
-- may be *monoclonal* (single peak): strong antigen selection is suggested
-- no product amplified for that BV family

Fig. 1. An outline of the methods used to analyze the clonality of a T cell population.

The spectratype approach demonstrates the polyclonal characteristics of a CD4 T cell population in peripheral blood, left frame. This distribution of β-chain lengths would be similar to that given by the entrance of T cells into tissue that is a secondary consequence of chemokines released by non-specific inflammation. The right frame illustrates a highly oligoclonal pattern exhibited by the memory-effector T cell subset ($CD8^+CD28^-$) in blood. This antigen-driven pattern is composed of two major clones found at 522 and 528 nucleotides. Often the nucleotide size is converted to the length in amino acids of the hypervariable region of the CDR3 between two benchmarks in the V and J segments. The V segment gene elements are grouped into 24 BV families and typically a panel of 24 primers, each specific for a BV family, is used to assess repertoire complexity. Figure 1 shows the BV8 family distribution of β-chain lengths.

2. The inflammatory infiltrate in bicuspid and tricuspid valve calcific aortic stenosis is characterized by clonal expansions of T cells

These methods of repertoire analysis can be used to characterize the inflammatory infiltrate in the aortic valve. The TCR β-chain CDR3 length distribution in valves removed at surgery was found to be highly restricted, with skewed length spectra indicating the presence of a considerable number of oligoclonal expansions, (Wu et al. 2007) as illustrated below in Figure 2. Despite considerable heterogeneity, the majority of the infiltrating T cells in most valves and in most BV families consist of oligoclonal expansions, as shown by patterns containing one, two, or three peaks similar to those illustrated. The mean Hamming distance (mHD) is a

statistical measure of the departure of the observed pattern of the valve repertoire from a reference polyclonal repertoire of control CD4 T cells from healthy individuals. The distance would be zero if the valve repertoire was identical to the reference, and 100 if it was completely different. The mHD across all expressed BV families was 60, range 45–80, illustrating the marked difference of the tricuspid aortic valve repertoire from the polyclonal reference repertoire ($p < 0.001$) and emphasizing the overall oligoclonal character of the T cell infiltrate in CAS valves (Winchester et al. 2011). Interestingly, some valves also had variable degree of polyclonal infiltration correlating with calcification severity. This same pattern of extensive oligoclonality and variable polyclonality was seen in bicuspid valves and their repertoire characteristics were indistinguishable from those of the tricuspid valves, Figure 2.

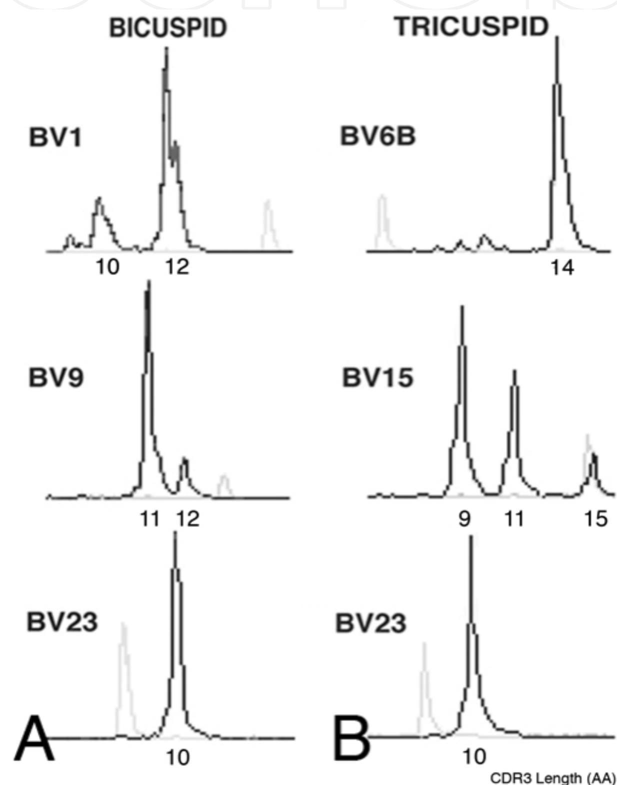


Fig. 2. Spectratype β-chain length distribution images illustrating the extensive oligoclonality in 3 BV families in a representative bicuspid (A) and tricuspid (B) CAS valve.

This counters the hypothesis that the repertoire of T cells infiltrating the valve leaflets is polyclonal and simply is a result of the presence of antecedent injury and inflammation. The presence of numerous clonal expansions in combination with a lesser, but variable degree of polyclonal infiltration argues for the interpretation that specific clonal expansions, likely induced by an adaptive immune response, are a component of CAS.

Since a few clones exhibited sequence homologies to clones previously identified in various inflammatory sites, such as multiple sclerosis lesions and HTLV-1 infection, (Wu et al. 2007), this suggests that a minority of CAS clones may be considered as bystander clones (McNally & Welsh 2002) related to the non antigen specific component of inflammation. However, the minor proportion of polyclonal, non-expanded T cells in many CAS samples compared to other inflammatory sites such as atherosclerotic plaques of inflamed synovia (Curran et al. 2004, Oksenberg et al. 1997, Stemme et al. 1991, Swanson et al. 1994), argues that inflammation-mediated T cell recruitment is not a general feature of CAS.

3. The inflammatory infiltrate in bicuspid and tricuspid valve calcific aortic stenosis is primarily associated with elevated proportions of circulating activated and effector memory CD8 T cells

The finding of clonal expansion in CAS valve leaflets raises the question of whether features of an ongoing systemic immune response would be present and be demonstrable in peripherally circulating T cells, implying that CAS had a systemic component. The alteration in T cell phenotype during an immune response, especially evident in the CD8 T cells, usually includes the transient expression of activation molecules, such as HLA-DR(Yu et al. 1980) and/or CD69, and the development of memory-effector cells particularly in sustained responses, defined by the loss of co-stimulatory CD28 molecules and acquisition of structures including natural killer receptors, e.g. CD57 (Brenchley et al. 2003, Hsu et al. 2006, Sallusto et al. 2004, Speiser et al. 1999). The presence of these features was studied by using flow cytometry to determine the expression of activation markers on the peripheral blood T cells and their subsets (Winchester et al. 2011). The combination of markers present can distinguish between naïve (CD28^{+} and CD57^{-}) and memory effector (CD28^{-} and CD57^{+}) T cells. Figure 3, next page, depicts four examples. Bicuspid valve sample B63 exhibits intense CD3 T cell activation as shown by the high proportion of T cells expressing HLA-DR, compared with a lower level in sample B48. The CD8 T cells of sample T54 exhibit extensive differentiation to CD28^{-} memory effector status as shown by loss of CD28, versus a lesser degree of differentiation in sample T55.

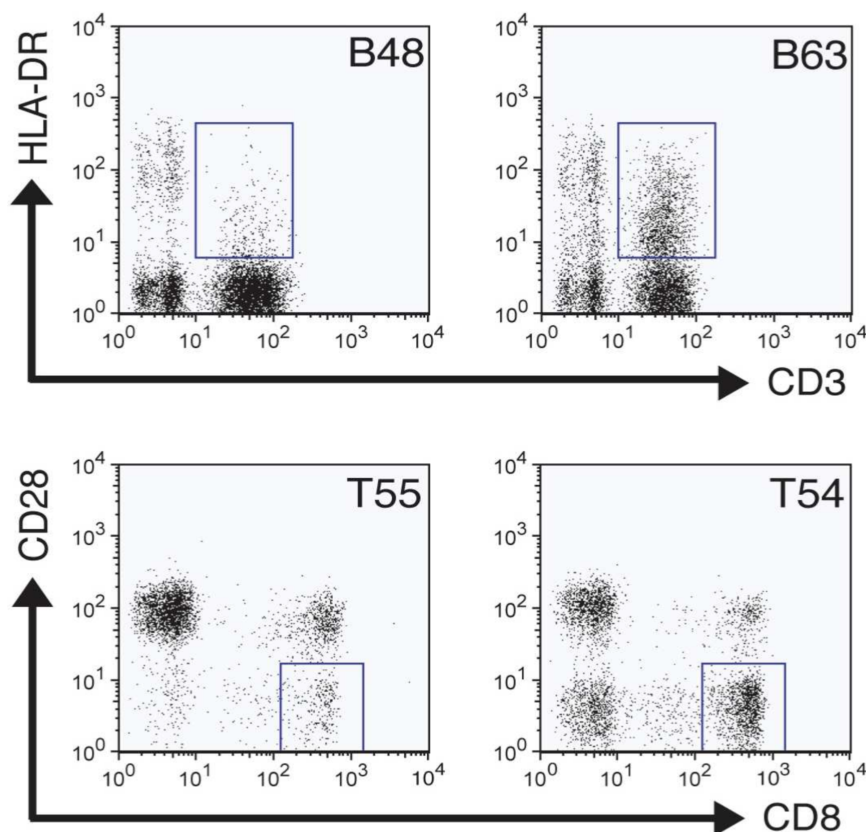


Fig. 3. Flow cytometry study of peripheral blood T lymphocytes from 4 CAS cases illustrates four different combinations of markers that identify activation (HLA-DR+) or varying degrees of differentiation to the CD28^{null} memory-effector phenotype (CD28-).

The proportion of circulating CD3+ T cells expressing HLA-DR in CAS was considerably increased in the peripheral T cell compartment, range 4.7 to 32.9%, mean 16.1% and 16.3% in cases with tricuspid and bicuspid CAS valves, respectively, compared to the expected frequency of ≤ 5% in healthy controls (Winchester et al. 2011). The proportion of activated HLA-DR+ T cells correlated with a semiquantitative calcification score ranging from 1 through 8, correlation, $\rho = 0.530$, $p = 0.024$. Interestingly the percentage of the $CD8^{+}CD57^{+}$ T cell subset expressing HLA-DR ranged up to 49.9% and was greater than that found on the entire CD8 T cell subset in 11 of 14 CAS cases, indicating the CD8 T cells that have differentiated to a memory-effector phenotype continue to be strongly activated.

The proportion of circulating $CD8^{+}$ T cells that extinguished expression of CD28, one of the main markers of differentiation to the memory-effector phenotype, was substantially increased in tricuspid CAS, range 36.6 to 96%, mean 69.7% of CD8 T cells, and similarly in bicuspid CAS, range 20 to 85.5%, mean 65.8% (Winchester et al. 2011). Among tricuspid CAS cases the percentage of $CD8^{+}CD28^{null}$ T cells correlated with valve calcification severity ($\rho = 0.666$, $p = 0.003$). In the much younger bicuspid CAS patients, mean age 56± 18 years, the proportion of $CD8^{+}CD28^{null}$ T cells was more than double the level expected in normal age-matched individuals (Hsu et al. 2006). For all valve types, a greater proportion of $CD8^{+}CD28^{null}$ T cells was seen in cases where the valve calcification severity score was ≥ 4 ($p = 0.0006$), and the correlation with calcification score was $\rho = 0.590$, $p = 0.001$.

Because some of the findings of differentiation to memory effector phenotype are associated with physiologic aging and have been used to argue for immunologic senescence, immunosenescence, it was critical to study CAS occurring in bicuspid aortic valve cases, which occurs in individuals that are several decades younger. The results in those with bicuspid aortic valves were entirely similar to those in tricuspid aortic valve stenosis, indicating that this immune response was an intrinsic feature of CAS, and not likely a consequence of aging of the immune system.

The marked elevations in the level of activated CD8 T cells ($HLA\text{-}DR^{+}$ $CD69^{+}$) and extensive differentiation to memory effector phenotype ($CD28^{-}CD57^{+}$) are features indicative of an intense systemic immune response. These elevations generally parallel the infiltration of the valve by clonally expanded T cell populations. Furthermore, the extent of activation and expansion of the memory effector subsets in CAS cases was directly and strongly correlated with CAS severity (Winchester et al. 2011). Taken together with the elevated proportion of cells exhibiting differentiation to memory effector phenotype, these findings imply that a systemic immune response accompanies CAS.

In approximately half of the cases a lesser degree of activation and differentiation to the memory effector phenotype was found in the circulating CD4 T cell subset. The percentage of $CD4^{+}CD28^{null}$ T cells was moderately elevated, mean = 14.8%, range 2.6 to 50.5%, with a significant difference in mean frequency of the CD4+CD28- T cell subset between the atherosclerotic positive (19.36%) and negative (6.94%) subsets, $p = 0.0074$. (Winchester et al. 2011). Implicating this subset in the process of CAS, the number of $CD4^{+}CD28^{null}$ T cells was increased among cases with extensive valvular calcification, CAS score ≥4 ($p = 0.007$).

3.1 Immunostaining of CAS valves

Immunostaining of the valves was performed to define the phenotype and distribution of infiltrating T cells to aid in the interpretation of the repertoire analyses and relating the

findings in the valve T cell infiltration to the phenotype found in blood (Winchester et al. 2011, Wu et al. 2007). The valve illustrated in figure 4 had a moderate level of polyclonality found by repertoire analysis, however upon staining, the large proportion of the infiltrating T cells exhibit the phenotype $CD8^{+}CD28^{-}$in the region of neovascularization. This is near a region of calcification at the lower left. There is considerable heterogeneity among different valves in terms of the number of infiltrating T cells and their immunophenotype found on immunostaining. Valves with multi or polyclonal T cell infiltration usually exhibited many regions of very abundant infiltration by CD8 and CD4 T cells. In contrast more oligoclonal samples had more sparse regions of T cell infiltration that predominantly stained for CD8. In all valves, the majority of CD8 T cells either lacked detectable expression of CD28 or staining was very dim, as shown in Figure 3. These $CD28^{null}$ or $CD28^{dim}CD8^{+}$ T cells were often found in proximity to sites of calcification or neovascularization, Figure 4. Reciprocally, in valves with greater proportions of polyclonal T cells, and with higher percentages of $CD4^{+}CD3^{+}$ T cells, more were $CD28^{brt}CD8^{-}$ (Winchester et al. 2011).

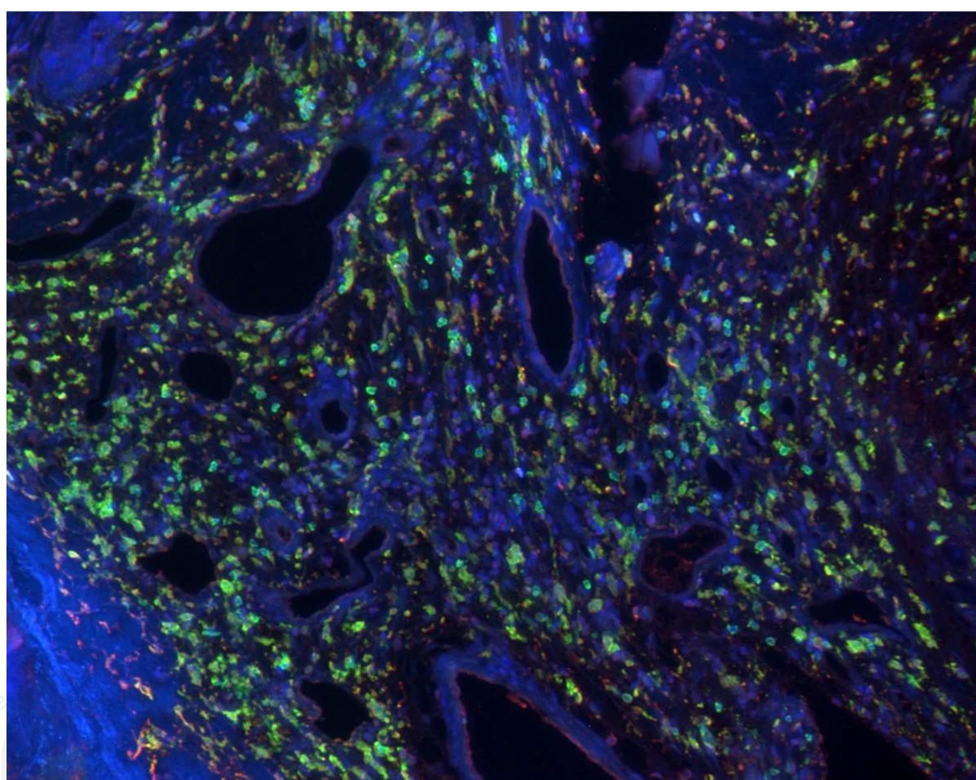


Fig. 4. An area of a tricuspid aortic valve exhibiting extensive neovascularization. It is stained for the expression of CD8 (green), CD28 (red-orange) and nuclei (Blue). The large proportion of the infiltrating T cells stain brightly for only the green fluorescence and have the phenotype $CD8^{+}CD28^{-}$.

4. Trafficking of the same T cell clones found expanded in the valve and T cell subsets in peripheral blood undergoing expansion

Trafficking of a subset of clones between the peripheral blood CD8 T cell subset and the infiltrate in a CAS valve is illustrated in figure 5 for three BV families. The darkened rectangles indicate that the same clone is found in the blood CD8 subset and in the valve.

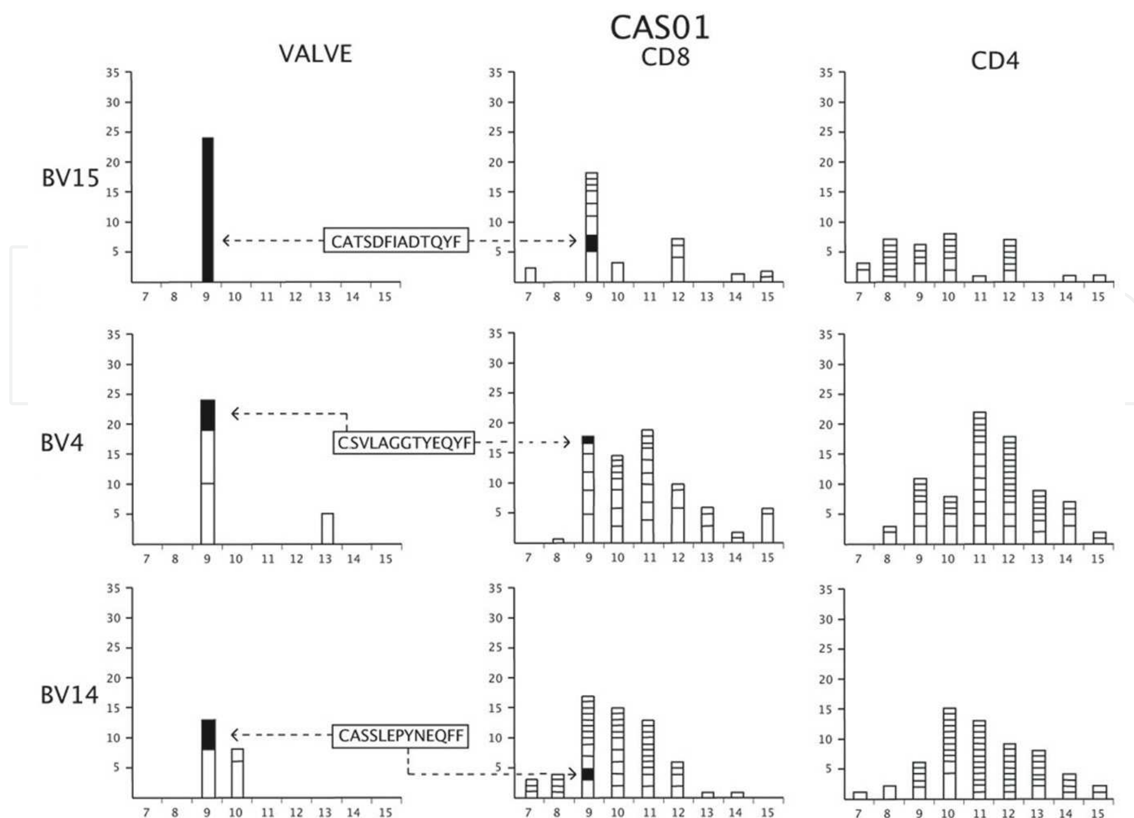


Fig. 5. An example of sharing of some clones expanded in the valve with the CD8 T cell subset. The repertoire analysis has been done by sequencing the TCR β-chain, and assembling the results into distribution histograms. The size of the rectangle denotes the clone size, with single sequence clones predominating in the CD4 subset.

In the initial study identifying clonal expansions in the valve, 24 of the valve-infiltrating T cell clones also had the same clone identified in blood. Importantly, 22 of these 24 shared clones were CD8 in lineage ($p=1.5x10^{-12}$) (Wu et al. 2007). As is seen in figure 5, in the valve, of the 9 expanded clones in the three examples, only three of these clones had clonal precursors identified in the blood, indicating evident clonal trafficking between the blood and the valve. Reciprocally a large number of clones are seen in the peripheral blood CD4 and CD8 subsets, of which only these 3 are found in the valve. This is an indication of the specificity of clonal entrance, and argues against passive streaming of clones from blood into the valve.

Further analysis and separation of the CD8 compartment of peripheral blood into the CD28+ naïve subset and the CD28- memory effector subset showed the large majority of T cell clones that were shared between the valve and the peripheral blood CD8 compartment were found in the memory effector $CD8^+CD28^{null}$ subset (Winchester et al. 2011), Table 1. Some clones were also shared with the CD8+CD28+ subset, and in each of these instances the clone was found expanded, indicating that it was proliferating and presumably in the process of differentiating to a memory effector phenotype. The clone with the clonotype sequence CASLALAFNEQFF, is an example of a clone in the process of this differentiation, and is found in both the CD8+CD28+ subset and the $CD8^+CD28^{null}$ subset (Winchester et al. 2011). These observations provide direct evidence supporting the association between the elevation in proportion of the $CD8^+CD28^{null}$ subset in blood and CAS severity. They show

that the systemic immune response associated with CAS is an intrinsic part of the valve inflammation.
Additionally, one shared, but not expanded, clone in the CD4 blood subset was found with the clonotypic sequence CASSSKRLAGESGELFF, in a case that also had atherosclerotic disease. This instance of a minimally expanded clone that is shared between blood and the valve might reflect participation of the atherosclerotic process.

Case	TRBV-CDR3	Clonotypic CDR3 sequence	Valve clone size	Blood Subset	Blood clone size
T13	TRBV7-10	CASSQAPGKAFF	8	$CD28^+$ $CD8^+$	4
T13	TRBV7-12	CASSLDTRGDTQYF	4	$CD28^{null}$ $CD8^+$	38
T13	TRBV7-15	CASSSKRLAGESGELFF	1	$CD4^+$	1
T13	TRBV12-13	CASSRTSGVYNEQFF	11	$CD28^{null}$ $CD8^+$	22
T14	TRBV11-12	CASSLALAFNEQFF	18	$CD28^{null}$ and	3
				$CD28^+$ $CD8^+$	4
T14	TRBV11-17	CASSLNDRGVGLSSYEQYF	2	$CD28^+$ $CD8^+$	11

CDR3 length, number of amino acids in CDR3 loop according to IMGT nomenclature
Clone size refers to number of sequence of this clonotype recovered in a set of 40 sequences

Table 1. Shared T cell clonotypes recovered from aortic valve and peripheral blood

5. Discussion

Three central findings emerged from these studies on the immunological nature and significance of the T lymphocytes that infiltrate the valve leaflets in CAS: First, as shown by TCR β–chain sequencing and spectratype analysis, the T cell infiltrate in the CAS valves predominantly consists of expanded αβ TCR clones, with a varying component polyclonal T cell infiltration. This finding suggests that a major component of the T cell infiltrate in the leaflets in CAS appears to be due to antigen-induced proliferation of T cells. The extent of the clonal expansions correlated with the severity of CAS. Immunostaining revealed a large, but variable proportion of the infiltrating T cells were CD8 in lineage and exhibited the memory effector phenotype of extinguishing expression of CD28, supporting the interpretation of antigen-induced differentiation. In addition to the clones some tissues exhibited a polyclonal infiltration of non-clonally expanded T cells indicative of non-antigen-specific inflammation and likely chemokine driven recruitment.
The second main finding was that the peripheral blood of CAS cases had greatly elevated levels of activated CD8 T cells ($HLA-DR^+$ and/or $CD69^+$) and extensive differentiation to memory effector phenotype ($CD28^-CD57^+$). These features in peripheral blood indicate an intense ongoing systemic immune response. The extent of activation and expansion of the peripheral blood memory effector subsets in CAS cases was directly and strongly correlated with CAS severity. The results in those with bicuspid CAS were entirely similar to those found in tricuspid CAS, indicating that this immune response was an intrinsic feature of CAS and not likely to be secondary to immunosenescence. It is possible that the significant elevations of CRP reported in CAS cases (Galante et al. 2001, Jeevanantham et al. 2007) is a constitutive component of the CD8 T cell activation and differentiation to a memory-effector phenotype, together reflecting the presence of a systemic adaptive immune response. A

lesser degree of activation and differentiation to the memory effector phenotype was found in the CD4 T cell subset in approximately half of the cases. This was highly correlated with the presence of atherosclerotic disease regardless of whether it appeared stable or not.

The third central finding was the demonstration that particular T cell clones found expanded within the valve were also expanded in peripheral blood, especially in the memory effector CD8 subset. This evidence of clonal trafficking between the blood and valve provides direct evidence linking events in blood to those in the valve and supports the association of the activation and expansion of the memory effector T cell subset in blood with the severity of CAS.

Several findings point to an important role of CD8 T cells and in particular the $CD8^{+}CD28^{null}$ T cells in CAS. They include identification of large numbers of T cells with this phenotype in the valve on immunostaining; activation and differentiation to memory-effector status among circulating lymphocytes, which strongly predominated in the CD8 T cell subset; as well as 8 instances of sharing of the same T cell clones expanded both in the valve and in the peripheral blood memory-effector $CD8^{+}CD28^{null}$ T cells (Winchester et al. 2011) Moreover, HLA-DR expression was particularly increased on the $CD8^{+}CD57^{+}$ T cell subset, indicating their continued activation. Together with the earlier findings where 23 of 24 clones identified both among the circulating lymphocytes and in the valve were CD8 lineage T cells (Wu et al. 2007) these data suggest the interpretation that the peptides driving this aspect of the immune response in CAS likely have a cytoplasmic origin, are presented in the context of class I MHC molecules and are recognized by CD8 T cells.

The selectivity of the process underlying the presence of the expanded T cells infiltrating the valve is supported by the absence of T cells from certain BV families in valve tissue (Winchester et al. 2011, Wu et al. 2007), the highly significant predominance of the CD8 subset among the clones shared with blood, the large proportion of clonal expansion in both blood CD4 and CD8 repertoires that were not identified in the valve and vice verse, the elevated proportion of expanded clones to unexpanded clones found in the valve, the structural homologies evident between CDR3 regions of unrelated clones as well as the frequent representation of the same CDR3 length in the valve (Wu et al. 2007). This selectivity is consistent with the operation of cognitive adaptive immune recognition events in the formation of the inflammatory infiltrate of the valve.

5.1 Atherosclerosis and CAS

CAS shares some risk factors with atherosclerosis and apoE knockout mice develop CAS (Ortlepp et al. 2003, Stewart et al. 1997, Tanaka et al. 2005). However, as emphasized by Otto et al.,(Otto & O'Brien 2001) CAS and atherosclerosis are not synonymous, given that only half of the patients with CAS have concomitant coronary artery disease, while the large majority of those with atherosclerosis do not develop CAS (Ortlepp et al. 2003). Additionally, bicuspid aortic valves are disproportionally affected with CAS further suggesting that factors distinct from atherosclerosis account for the development of CAS (Otto 2002). Moreover, the lack of clear efficacy of intensive lipid lowering therapy by statins in halting the progression of CAS (Cowell et al. 2005) suggests that processes other than those involved in atherosclerosis may be at play in CAS.

These facts notwithstanding, there is indirect evidence from our work that in CAS cases with evident atherosclerosis that the atherosclerotic process interacts with mechanisms responsible for CAS. Elevations in the $CD28^{null}$ CD4 T cell subset are well recognized in

unstable atherosclerotic disease (Liuzzo et al. 2000). In CAS peripheral blood, as discussed there was a significant difference in mean frequency of the CD4+CD28- T cell subset between the atherosclerotic positive (19.36%) and negative (6.94%) subsets, p= 0.0074. (Winchester et al. 2011). Implicating this subset in the process of CAS, the number of $CD4^{+}CD28^{null}$ T cells was increased among cases with extensive valvular calcification. Additionally, one instance of a shared, but not detectably expanded, clone between the CD4 blood subset was found (Table 1), in a case that also had atherosclerotic disease. It is possible this instance of a shared T cell clone reflects the contribution of the co-morbid atherosclerotic process (Oksenberg et al. 1997, Stemme et al. 1991). However, overt unstable atherosclerotic disease was not present in the CAS cases studied by us, suggesting that if the $CD4^{+}CD28^{null}$ T cell elevations in the subset of CAS cases with atherosclerotic is mechanistically linked to the atherosclerotic process, the role of this cellular subset in CAS differs from that proposed in unstable atherosclerosis. Moreover, as emphasized by Wu et al. (Wu et al. 2007), the overall oligoclonal nature and CD8 lineage of valve-infiltrating T cells and those expanded in blood that predominate in CAS contrast sharply with the findings reported in atherosclerosis (Oksenberg et al. 1997, Stemme et al. 1991). This underlines the fact that the predominant immune mechanism underlying the lymphocytic infiltration in CAS centered on CD8 T cells is distinct from that implicated in atherosclerosis.

5.2 Immunosenescence

In normal chronologic aging the prevalence of $CD28^{null}CD57^{+}$ T cells increases in the $CD8^{+}$ and, to a much lesser extent, in the $CD4^{+}$ compartment (Effros et al. 1994) along with large clonal expansions in this subset (Bandres et al. 2000, Posnett et al. 1994, Vallejo 2006). These expansions have been attributed in part to the sustained proliferation of $CD8^{+}$ T cells involved in maintaining viral latency to EBV and in responding to CMV. Effros and colleagues advanced the concept that $CD8^{+}CD28^{null}$ T cells are immunosenescent and that their expansion primarily down modulates both immune and non-immune functions and contributes to various age-related pathologies. Intriguingly in one sense, the present findings in tricuspid CAS are generally consistent with the association noted by Effros et al., especially given the advanced age of the tricuspid CAS cases, and in this respect tricuspid CAS can be added to conditions characterized by expansions of memory-effector T cells. (Effros et al. 2005, Goronzy & Weyand 2003, Hadrup et al. 2006, Hamann et al. 1999, Morley et al. 1995, Posnett et al. 1999, Posnett et al. 1994).

However, these data can be interpreted differently and studies presented in this chapter support the viewpoint that the mechanism differs from that proposed by Effros et al. in that CAS is not a secondary consequence of immunosenescence. The recent studies discussed in this chapter are consistent with the opposite possibility that a specific immune response involving the valve leaflets drives the activation and expansion of CD8 T cells and their maturation to $CD28^{null}$ phenotype. Accordingly we envision the expansion of the memory-effector subset to be a consequence of and a component of the immune response directed to the valve. This interpretation is supported by: a) The high expression of HLA-DR and CD69 on $CD8^{+}CD28^{null}$ T cells in CAS that distinguishes this condition from the $CD8^{+}CD28^{null}$ T cell populations in aging; b) The extent of the expansion of the $CD28^{null}CD8^{+}$subset that was directly and significantly correlated with the CAS severity score (p=0.003), but was independent of chronologic age; and c) Perhaps most consequentially, the complete resemblance of the findings in blood and in the valve between the much younger bicuspid

valve CAS cases and the older tricuspid valve cases, emphasizing the importance of studying the much younger individuals who develop CAS in bicuspid aortic valves.

6. Conclusion

The results suggest that an ongoing systemic adaptive immune response is occurring in cases with bicuspid and tricuspid CAS, involving circulating CD8 T cell activation, clonal expansion and differentiation to a memory-effector phenotype, with trafficking of T cells in expanded clones between blood and the valve. The character of the immune response and the primary involvement of CD8 T cells suggests the T cell response is likely driven by a cellularly expressed antigen present in the valve leaflet, likely present in the cytoplasm of leaflet mesenchymal cells. The present studies raise several additional questions.

The first major question arises of what immune recognition event might be driving these immune events. Because CAS occurs in the setting of different clinical situations of bicuspid and tricuspid aortic valves and co morbidities including hypertension and atherosclerosis that have in common enhanced hemodynamic strain and diminished valvular compliance, one unifying hypothesis is that valve mesenchymal cells alter their transcriptional phenotype in response to enhanced strain, resulting in the cellular expression of stress-induced molecules. Peptides from these stress-induced molecules, to which the individual may not be fully tolerized, may be expressed in the context of class I MHC inducing an idiosyncratic autoimmune-like CD8 T cell immune response. This results in infiltration of the valve by expanding and activated T cell clones that recognize the stress neoantigen. Of course as in all autoimmune diseases the adaptive immune response to a self-antigen is not appreciably different from one to a pathogen, and the present studies leave open a range of possibilities as to the source and nature of the peptides driving this immune response. For several reasons, identification of the peptides recognized by the infiltrating T cell clones would be a central advance.

Since this hypothesis considers the expression of stress induced antigens in the valve as the driver of the systemic immune response identified in CAS cases, one approach to testing the hypothesis is to examine the effects of valve replacement surgery on the extent of the circulating CD8 T cell activation and differentiation to memory effector status. The hypothesis suggests that the level of activation and differentiation should return towards more normal levels. These studies are currently underway.

A second major question is what is the relevance of this immune response to CAS? It is possible these proliferating T cells release cytokines that alter the pattern of gene expression in the valve mesenchymal cells and recruit additional lymphocytes into the valve, suggesting that at a minimum one consequence of this inflammation is a further and potentially reversible decrease in valvular compliance. Support for this possibility comes in part from preliminary data in 8 cases where significant production of γ-interferon was found in the $CD8^{+}CD28^{null}$ T cell subset and from the earlier reports identifying HLA-DR expression on valve mesenchymal cells (Olsson, Rosenqvist, et al. 1994). This would be a positive feedback situation where the consequence of diminished valve compliance would presumably enhance the stress response of the mesenchymal cell leading to enhanced expression of stress antigens. It is also possible that the activated memory-effector CD8 T cells directly injure target valve cells and may additionally drive the development of heterotopic calcification, and neovascularization with precedent for development of

analogous heterotopic calcification in the setting of the autoimmune CD8^{+}CD28^{null} T cell response in diseases like psoriatic arthritis or ankylosing spondylitis.
Another major question is whether the extent of immune reactivity in blood could be a marker for the rate of progression of CAS, since both the extent of the changes in the blood and the infiltration in the valve vary considerably. However, the cross sectional nature of this study that demonstrated a correlation between intensity of CD8 T cell activation and differentiation to memory effector phenotype with calcification severity does not allow assessment of the predictive value of these measurements for the development of severe CAS. It is possible that low levels of CD8 T cell activation and differentiation to memory effector phenotype could be found to be a biomarker favoring slow progression of calcification and other features of the process. The use of tetramer technology with knowledge of the nature of the driving peptide would be the ideal way to specifically assess the intensity of this immune response and distinguish it from other CD8 T cell responses.
This line of immunologically oriented research work should add impetus to changing the view of CAS as an irreversible degenerative process, set the stage for identification of the target antigens driving this process, and give hope to the possibility of designing specific immunomodulatory therapy directed towards inhibiting the T cell clonal expansions or lymphocyte recruitment to diminish the relentless progression of this serious disease, for which currently valve replacement is the only treatment.

7. Acknowledgement

We are indebted to the many colleagues who have provided well-defined CAS cases and tissue samples for study, and who have contributed to data acquisition and analysis. We gratefully acknowledge the support of the USPHS, grant number R01HL084599.

8. References

Bandres, E., Merino, J., Vazquez, B., Inoges, S., Moreno, C., Subira, M. L. & Sanchez-Ibarrola, A. (2000) The increase of IFN-gamma production through aging correlates with the expanded CD8(+high)CD28(-)CD57(+) subpopulation. *Clin Immunol 96*(3): 230-235.

Brenchley, J. M., Karandikar, N. J., Betts, M. R., Ambrozak, D. R., Hill, B. J., Crotty, L. E., Casazza, J. P., Kuruppu, J., Migueles, S. A., Connors, M., Roederer, M., Douek, D. C. & Koup, R. A. (2003) Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood 101*(7): 2711-2720.

Cowell, S. J., Newby, D. E., Prescott, R. J., Bloomfield, P., Reid, J., Northridge, D. B. & Boon, N. A. (2005) A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med 352*(23): 2389-2397.

Curran, S. A., FitzGerald, O. M., Costello, P. J., Selby, J. M., Kane, D. J., Bresnihan, B. & Winchester, R. (2004) Nucleotide sequencing of psoriatic arthritis tissue before and during methotrexate administration reveals a complex inflammatory T cell infiltrate with very few clones exhibiting features that suggest they drive the inflammatory process by recognizing autoantigens. *J Immunol 172*(3): 1935-1944.

Effros, R. B., Boucher, N., Porter, V., Zhu, X., Spaulding, C., Walford, R. L., Kronenberg, M., Cohen, D. & Schächter, F. (1994) Decline in CD28+ T cells in centenarians and in long-term T cell cultures: a possible cause for both in vivo and in vitro immunosenescence. *Exp Gerontol 29*(6): 601-609.

Effros, R. B., Dagarag, M., Spaulding, C. & Man, J. (2005) The role of CD8+ T-cell replicative senescence in human aging. *Immunol Rev 205*: 147-157.

Galante, A., Pietroiusti, A., Vellini, M., Piccolo, P., Possati, G., De Bonis, M., Grillo, R. L., Fontana, C. & Favalli, C. (2001) C-reactive protein is increased in patients with degenerative aortic valvular stenosis. *Journal of the American College of Cardiology 38*(4): 1078-1082.

Goronzy, J. J. & Weyand, C. M. (2003) Aging, autoimmunity and arthritis: T-cell senescence and contraction of T-cell repertoire diversity - catalysts of autoimmunity and chronic inflammation. *Arthritis Res Ther 5*(5): 225-234.

Hadrup, S. R., Strindhall, J., Kollgaard, T., Seremet, T., Johansson, B., Pawelec, G., thor Straten, P. & Wikby, A. (2006) Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. *J Immunol 176*(4): 2645-2653.

Hamann, D., Kostense, S., Wolthers, K. C., Otto, S. A., Baars, P. A., Miedema, F. & van Lier, R. A. (1999) Evidence that human CD8+CD45RA+CD27- cells are induced by antigen and evolve through extensive rounds of division. *Int Immunol 11*(7): 1027-1033.

Hsu, H. C., Scott, D. K., Zhang, P., Zhou, J., Yang, P., Wu, Q., Schroeder, H. W., Jr., Gerald, L. B., Ravussin, E., Jazwinski, S. M. & Mountz, J. D. (2006) CD8 T-cell immune phenotype of successful aging. *Mech Ageing Dev 127*(3): 231-239.

Jeevanantham, V., Singh, N., Izuora, K., D'Souza, J. P. & Hsi, D. H. (2007) Correlation of high sensitivity C-reactive protein and calcific aortic valve disease. *Mayo Clinic proceedings. Mayo Clinic 82*(2): 171-174.

Li, Y., Schwabe, R. F., DeVries-Seimon, T., Yao, P. M., Gerbod-Giannone, M. C., Tall, A. R., Davis, R. J., Flavell, R., Brenner, D. A. & Tabas, I. (2005) Free cholesterol-loaded macrophages are an abundant source of tumor necrosis factor-alpha and interleukin-6: model of NF-kappaB- and map kinase-dependent inflammation in advanced atherosclerosis. *J Biol Chem 280*(23): 21763-21772.

Liuzzo, G., Goronzy, J. J., Yang, H., Kopecky, S. L., Holmes, D. R., Frye, R. L. & Weyand, C. M. (2000) Monoclonal T-cell proliferation and plaque instability in acute coronary syndromes. *Circulation 101*(25): 2883-2888.

Mazzone, A., Epistolato, M. C., De Caterina, R., Storti, S., Vittorini, S., Sbrana, S., Gianetti, J., Bevilacqua, S., Glauber, M., Biagini, A. & Tanganelli, P. (2004) Neoangiogenesis, T-lymphocyte infiltration, and heat shock protein-60 are biological hallmarks of an immunomediated inflammatory process in end-stage calcified aortic valve stenosis. *J Am Coll Cardiol 43*(9): 1670-1676.

McNally, J. M. & Welsh, R. M. (2002) Bystander T cell activation and attrition. *Curr Top Microbiol Immunol 263*: 29-41.

Morley, J. K., Batliwalla, F. M., Hingorani, R. & Gregersen, P. K. (1995) Oligoclonal CD8+ T cells are preferentially expanded in the CD57+ subset. *J Immunol 154*(11): 6182-6190.

Oksenberg, J. R., Stavri, G. T., Jeong, M. C., Garovoy, N., Salisbury, J. R. & Erusalimsky, J. D. (1997) Analysis of the T-cell receptor repertoire in human atherosclerosis. *Cardiovasc Res 36*(2): 256-267.

Olsson, M., Dalsgaard, C. J., Haegerstrand, A., Rosenqvist, M., Ryden, L. & Nilsson, J. (1994) Accumulation of T lymphocytes and expression of interleukin-2 receptors in nonrheumatic stenotic aortic valves. *J Am Coll Cardiol 23*(5): 1162-1170.

Olsson, M., Rosenqvist, M. & Nilsson, J. (1994) Expression of HLA-DR antigen and smooth muscle cell differentiation markers by valvular fibroblasts in degenerative aortic stenosis. *J Am Coll Cardiol 24*(7): 1664-1671.

Ortlepp, J. R., Schmitz, F., Bozoglu, T., Hanrath, P. & Hoffmann, R. (2003) Cardiovascular risk factors in patients with aortic stenosis predict prevalence of coronary artery disease but not of aortic stenosis: an angiographic pair matched case-control study. *Heart 89*(9): 1019-1022.

Otto, C. M. (2002) Calcification of bicuspid aortic valves. *Heart 88*(4): 321-322.

Otto, C. M., Kuusisto, J., Reichenbach, D. D., Gown, A. M. & O'Brien, K. D. (1994) Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation 90*(2): 844-853.

Otto, C. M. & O'Brien, K. D. (2001) Why is there discordance between calcific aortic stenosis and coronary artery disease? *Heart 85*(6): 601-602.

Pomerance, A. (1972) Pathogenesis of aortic stenosis and its relation to age. *Br Heart J 34*(6): 569-574.

Posnett, D. N., Edinger, J. W., Manavalan, J. S., Irwin, C. & Marodon, G. (1999) Differentiation of human CD8 T cells: implications for in vivo persistence of CD8+ CD28- cytotoxic effector clones. *Int Immunol 11*(2): 229-241.

Posnett, D. N., Sinha, R., Kabak, S. & Russo, C. (1994) Clonal populations of T cells in normal elderly humans: the T cell equivalent to "benign monoclonal gammapathy". *J Exp Med 179*(2): 609-618.

Rajamannan, N. M., Subramaniam, M., Rickard, D., Stock, S. R., Donovan, J., Springett, M., Orszulak, T., Fullerton, D. A., Tajik, A. J., Bonow, R. O. & Spelsberg, T. (2003) Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation 107*(17): 2181-2184.

Raulet, D. H. (2003) Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol 3*(10): 781-790.

Sallusto, F., Geginat, J. & Lanzavecchia, A. (2004) Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol 22*: 745-763.

Soini, Y., Salo, T. & Satta, J. (2003) Angiogenesis is involved in the pathogenesis of nonrheumatic aortic valve stenosis. *Hum Pathol 34*(8): 756-763.

Speiser, D. E., Valmori, D., Rimoldi, D., Pittet, M. J., Lienard, D., Cerundolo, V., MacDonald, H. R., Cerottini, J. C. & Romero, P. (1999) CD28-negative cytolytic effector T cells frequently express NK receptors and are present at variable proportions in circulating lymphocytes from healthy donors and melanoma patients. *Eur J Immunol 29*(6): 1990-1999.

Stemme, S., Rymo, L. & Hansson, G. K. (1991) Polyclonal origin of T lymphocytes in human atherosclerotic plaques. *Lab Invest 65*(6): 654-660.

Stewart, B. F., Siscovick, D., Lind, B. K., Gardin, J. M., Gottdiener, J. S., Smith, V. E., Kitzman, D. W. & Otto, C. M. (1997) Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol 29*(3): 630-634.

Swanson, S. J., Rosenzweig, A., Seidman, J. G. & Libby, P. (1994) Diversity of T-cell antigen receptor V beta gene utilization in advanced human atheroma. *Arterioscler Thromb 14*(7): 1210-1214.

Tanaka, K., Sata, M., Fukuda, D., Suematsu, Y., Motomura, N., Takamoto, S., Hirata, Y. & Nagai, R. (2005) Age-associated aortic stenosis in apolipoprotein E-deficient mice. *J Am Coll Cardiol 46*(1): 134-141.

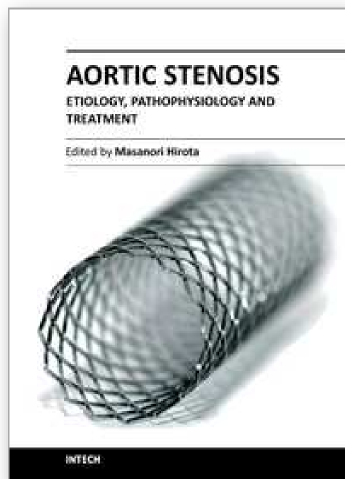
Vallejo, A. N. (2006) Age-dependent alterations of the T cell repertoire and functional diversity of T cells of the aged. *Immunol Res 36*(1-3): 221-228.

Wallby, L., Janerot-Sjoberg, B., Steffensen, T. & Broqvist, M. (2002) T lymphocyte infiltration in non-rheumatic aortic stenosis: a comparative descriptive study between tricuspid and bicuspid aortic valves. *Heart 88*(4): 348-351.

Winchester, R., Wiesendanger, M., O Brien, W., Zhang, H.-Z., Maurer, M. S., Gillam, L. D., Schwartz, A., Marboe, C. & Stewart, A. S. (2011) Circulating activated and effector memory T cells are associated with calcification and clonal expansions in bicuspid and tricuspid valves of calcific aortic stenosis. *Journal of Immunology (In Press)*.

Wu, H. D., Maurer, M., Friedman, R. A., Marboe, C. C., Ruiz-Vazquez, E. M., Ramakrishnan, R., Schwartz, A., Tilson, M. D., Stuart, A. S. & Winchester, R. (2007) The Lymphocytic Infiltration In Calcific Aortic Stenosis Predominantly Consists of Clonally Expanded T Cells. *Journal of Immunology 178*: 5329-5339.

Yu, D. T., Winchester, R. J., Fu, S. M., Gibofsky, A., Ko, H. S. & Kunkel, H. G. (1980) Peripheral blood Ia-positive T cells. Increases in certain diseases and after immunization. *Journal of Experimental Medicine 151*: 91-100.



Aortic Stenosis - Etiology, Pathophysiology and Treatment
Edited by Dr. Masanori Hirota

ISBN 978-953-307-660-7
Hard cover, 254 pages
Publisher InTech
Published online 10, October, 2011
Published in print edition October, 2011

Currently, aortic stenosis (AS) is the most prevalent valvular disease in developed countries. Pathological and molecular mechanisms of AS have been investigated in many aspects. And new therapeutic devices such as transcatheter aortic valve implantation have been developed as a less invasive treatment for high-risk patients. Due to advanced prevalent age of AS, further discovery and technology are required to treat elderly patients for longer life expectancy. This book is an effort to present an up-to-date account of existing knowledge, involving recent development in this field. Various opinion leaders described details of established knowledge or newly recognized advances associated with diagnosis, treatment and mechanism. Thus, this book will enable close intercommunication to another field and collaboration technology for new devices. We hope that it will be an important source, not only for clinicians, but also for general practitioners, contributing to development of better therapeutic adjuncts in the future.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Robert Winchester and Susheel Kodali (2011). The Inflammatory Infiltrate in Calcific Aortic Stenosis is Characterized by Clonal Expansions of T Cells and is Associated with Elevated Proportions of Circulating Activated and Effector Memory CD8 T Cells, Aortic Stenosis - Etiology, Pathophysiology and Treatment, Dr. Masanori Hirota (Ed.), ISBN: 978-953-307-660-7, InTech, Available from:
http://www.intechopen.com/books/aortic-stenosis-etiology-pathophysiology-and-treatment/the-inflammatory-infiltrate-in-calcific-aortic-stenosis-is-characterized-by-clonal-expansions-of-t-c

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.