# Mls-1 and Mls-2 superantigens do not control susceptibility to collagen-induced arthritis in $H_I$ and $H_{II}$ mice

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## SUMMARY

The $H_I$ mouse line is sensitive to collagen-induced arthritis (CIA), whereas $H_{II}$ is refractory, although both express the H-$2^q$ permissive haplotype. The two lines also share the same T-cell receptor (TcR) gene haplotypes for $\alpha$ and $\beta$ chains. The distribution of mouse mammary tumour viruses (MMTV), which encode endogenous superantigens (SAg) such as minor leucocyte-stimulating antigens (Mls) known to modulate the available TcR-V$\beta$ repertoire, was investigated in the two lines. Mls-1 is present in $H_I$-susceptible mice, while Mls-2 and Mls-2-like SAg are absent in both lines. This suggests that Mls antigens play no significant role in the resistance to CIA. Moreover, $H_I$ and $H_{II}$ exhibit close V$\beta$ gene usage as assessed by fluorescence staining with 11 V$\beta$-specific monoclonal antibodies (mAb). These results indicate that mechanisms other than clonal deletion based on V$\beta$ expression and induced by SAg are involved in the resistance of H-$2^q$-positive mice to experimental arthritis. Yet, a slightly reduced level of V$\beta5^+$ T cells is observed in $H_{II}$ animals which might correlate with the presence of *Mtv-6* and *Mtv-9* proviruses.

Collagen-induced arthritis (CIA) is an autoimmune disease that develops in mice after injection of type II collagen (CII). Susceptibility to the disease is, in part, linked to the major histocompatibility complex (MHC). Only H-$2^q$ and H-$2^r$ mice are sensitive.[1] T lymphocytes play a crucial role in the generation of the disease since CIA cannot be induced in athymic nude mice. In addition, T cells from CII-immunized animals or CII-specific T-cell lines and clones can transfer the disease. Finally, injection of CD4- or T-cell receptor (TcR)-specific monoclonal antibodies (mAb) can prevent CIA.[2]

Identification of several mouse strains resistant to CIA, although expressing a permissive H-2 haplotype, has revealed that genes located outside the MHC are involved in the genetic control of susceptibility to CIA. SWR and AU/SS J (H-$2^q$) like RIII S/J (H-$2^r$) have genomic deletions of many V$\beta$ genes.[3,4] A correlation between these deletions and resistance to CIA has thus been suggested.[5] However, several segregation experiments have not confirmed this conclusion.[6,7] Moreover, [SWR→DBA/1] chimeras can develop the disease whereas [DBA/1→SWR] chimeras can not. Since DBA/1 (H-$2^q$) and SWR are, respectively, sensitive and resistant to CIA, these results suggest that lymphoid cells from SWR are able to induce CIA and that elements of the genetic background, distinct from V$\beta$ structural genes, participate in the control of the disease.[8]

The V$\beta$ repertoire can also be modulated by endogenous superantigens (SAg) such as those encoded by mouse mammary tumour viruses (MMTV) which can induce the deletion of T lymphocytes expressing particular V$\beta$ segments in mice with permissive MHC class II molecules.[9] For instance, Mls-1 SAg is encoded by *Mtv-7* and is recognized by T cells expressing V$\beta$6, V$\beta$7, V$\beta$8.1 or V$\beta$9 gene segments, whereas Mls-2 and Mls-2-like SAg, specific for V$\beta$3-bearing T cells, are the products of *Mtv-13* and *Mtv-1*, respectively (Table 1). Recently, it has thus been proposed that Mls-1 and, to a lesser extent Mls-2 and Mls-2-like SAg, would reduce CIA incidence by affecting the available V$\beta$ T-cell repertoire.[10]

$H_I$ and $H_{II}$ are two mouse lines genetically selected for high antibody (Ab) response to T-cell-dependent antigens (Ag) and share the same H-$2^q$ haplotype. Although both lines develop humoral response to CII, only $H_I$ is sensitive to CIA.[11] In previous works, we showed that these lines have an identical genomic organization of their TcR-$\alpha$ and $\beta$ gene complex with no deletion of V$\beta$ segment.[12,13]

To explore the putative influence of Mls SAg on the susceptibility to CIA, we investigated MMTV distribution in $H_I$ and $H_{II}$ lines by Southern blot analysis on genomic DNA upon *Pvu*II and *Eco*RI digestion.[14] An ubiquitous *Mtv-C3H* LTR probe and a probe specific for the *env* 3′ end, isolated from *Mtv-8*, were used to detect proviruses. Identification of MMTV was

Abbreviations: CIA, collagen-induced arthritis; CII, type II collagen; MHC, major histocompatibility complex; Mls, minor leucocyte-stimulating antigens; MMTV, mouse mammary tumour virus; SAg, superantigens; TcR, T-cell receptor.

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**Table 1.** MMTV distribution in $H_I$ and $H_{II}$ lines

| *Mtv-** | *1* | *3* | *6* | *7* | *8* | *9* | *11* | *13* | *27* | *43* | *44* | *50* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mls† | 2-like | — | 3 | 1 | — | — | — | 2 | — | — | — | — |
| Vβ affected‡ | 3 | 3 | 3, 5.1, 5.2 | 6, 7, 8.1, 9 | 11 | 5.1, 5.2, 11 | 11 | 3 | 3 | 6, 7, 8.1, 9 | 3, 6, 8.1, 9 | 6, 8.1 |
| $H_I$ | −§ | − | − | + | − | − | − | − | − | − | − | − |
| $H_{II}$ | − | − | + | − | + | + | − | − | − | − | − | − |

* Only MMTV with known deletary effects on the T-cell Vβ repertoire are considered.
† Mls nomenclature.
‡ Data compiled from literature.
§-, MMTV absent; +, MMTV present.

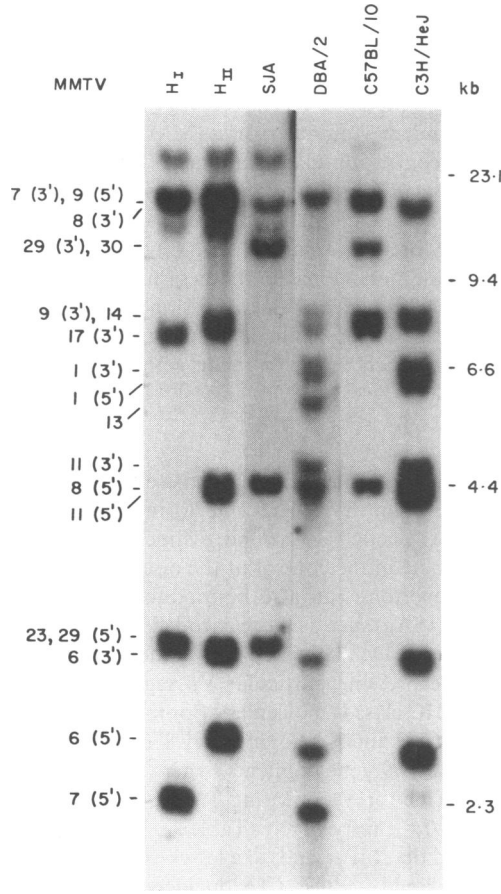


**Figure 1.** Southern blot analysis of MMTV integration in $H_I$ and $H_{II}$ mice. DNA was digested with *Pvu*II. Hybridization was performed using a *Mtv-C3H* LTR probe. Fragment identity, on the left, was inferred by comparison with patterns observed within C3H/HeJ, C57BL/10, DBA/2 and SJA mice. Numbers on the right represent the size in kb of molecular standards (λ*Hin*dIII).

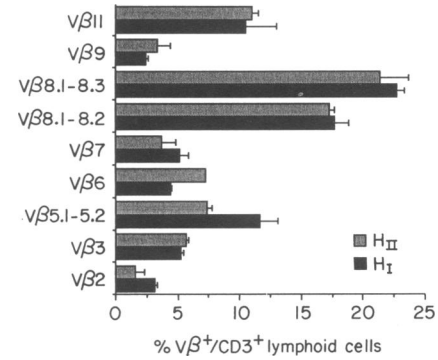


**Figure 2.** Expression of Vβ gene segments by peripheral T lymphocytes (lymph node $CD3^+$ cells).

deduced from the size of the fragments by comparison with those reported in the literature.

As shown on Fig. 1 and summarized in Table 1, $H_I$ but not $H_{II}$ mice bear *Mtv-7* encoding Mls-1. In addition, none of these mice expresses *Mtv-13 or Mtv-1* encoding Mls-2 and Mls-2-like SAg. Since $H_{II}$ are resistant to CIA, these results suggest that Mls-1, Mls-2 or Mls-2-like SAg are unlikely to protect against the disease. However, $H_{II}$ bear additional proviruses with known deletary effects on the expressed T-cell repertoire: *Mtv-6*, *Mtv-8* and *Mtv-9*, which are recognized by T cells expressing Vβ3, Vβ5.1-2 and Vβ11 (Table 1).

Recognition of endogenous SAg is strongly associated with the I-E molecules and to a lesser extent with I-A.[10] Consequently, clonal deletion of Vβ-bearing T cells mediated by endogenous SAg principally occurs in I-E-expressing animals. To test whether differential distribution of MMTV between $I\text{-}E^-$ $H_I$ and $H_{II}$ lines affects the Vβ repertoire, we determined the expression of 11 Vβ segments on T-cell surface by two-colour fluorescence analysis using mAb obtained from the following sources: Vβ2 (B20.6.5), B. Malissen (Marseilles, France); Vβ3 (KJ25) and Vβ8.1-8.2 (KJ16), P. Marrack and J. Kappler (Denver, CO); Vβ5 (MR 9.4), E. Palmer (Denver, CO); Vβ6 (44.22.1), H. Hentgartner (Zurich, Switzerland); Vβ7 (TR310) and Vβ9 (MR-10.2), Pharmingen (San Diego, CA); Vβ8.1-8.3 (F23.1), M. Bevan (Seattle, WA); and Vβ11 (KT11), K. Tomonari (London, U.K.). Anti-CD3 (145-2C11) was obtained from J. Bluestone (Chicago, IL). Lymph node cells of three $H_I$ and three $H_{II}$ mice were incubated with biotinylated anti-Vβ mAb and FITC-labelled anti-CD3. Vβ expression was revealed with streptavidin-PE (Becton Dickinson, Mountain View, CA). Fluorescence staining of $10^4$ cells was analysed using a FACStar® cell sorter (Becton Dickinson).

As indicated in Fig. 2, the two lines have a close usage of the different Vβ segments with no deletion of any particular Vβ-expressing T-cell subset. Yet, $H_I$ express half as much $V\beta6^+$ T cells as $H_{II}$ ($4{\cdot}4 \pm 0{\cdot}1\%$, and $7{\cdot}2 \pm 0{\cdot}1\%$, respectively; $P < 0{\cdot}001$),

a difference that could be attributed to the presence of *Mtv-7* in these mice.[10] Conversely, $H_I$ express more $V\beta5^+$ T lymphocytes than $H_{II}$ ($H_I$: $11{\cdot}7 \pm 1{\cdot}4\%$; $H_{II}$ $7{\cdot}4 \pm 0{\cdot}4\%$; $0{\cdot}01 > P > 0{\cdot}001$). This modulation most probably results from the presence of *Mtv-6* and *Mtv-9* in $H_{II}$ mice.[10]

Altogether, results reported herein indicate that expression of Mls-1, Mls-2 and Mls-2-like SAg can not account for differential susceptibility to CIA between $H_I$ and $H_{II}$ mice. Yet, although these lines express very close TcR-V$\beta$ repertoires, the slight decrease in $V\beta5^+$ T cells observed in $H_{II}$ mice could be mediated by *Mtv-6* and *Mtv-9* products and be involved in the resistance to CIA. This hypothesis should be confirmed by demonstrating that $V\beta5^+$ T cells contain an arthritogenic subset in $H_I$ mice that would be absent in $H_{II}$, although the two lines have different genetic backgrounds. Consistently, a limited V$\beta$ repertoire arises during the induction of CIA in DBA/1, which includes $V\beta5$ gene segments, and can be blocked partially or completely by anti TcR$\beta$–Ab therapy.[15] However, these blocking experiments, performed in adult animals in which the T-cell repertoire is established, do not help explain resistance of SWR or $H_{II}$ mice to CIA.

Hence, neither Mls nor TcR gene polymorphism seems sufficient to control susceptibility to CIA. Other genes should be involved, such as genes controlling CII processing or genes encoding for the C5 component of complement, particular V$\alpha$ segments, a CII allele or a CII cross-reacting antigen. Finally, tumour necrosis factor-$\alpha$ seems to play a central role in the development of CIA.[16,17] Endogenous factors might thus control the lymphokine produced upon injection of CII in complete Freund's adjuvant and subsequent breakdown of CII-autoreactive T-cell anergy.

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