Effect of Group 1 CD1-restricted T cells on Atherosclerosis

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INTRODUCTION

Approximately 1 in 3 deaths in the US is caused by cardiovascular diseases like atherosclerosis. Atherosclerosis occurs through excessive cholesterol deposition (hyperlipidemia) along the inner layer of the artery called the intima, resulting in plaque formation that blocks arterial blood flow and leading to heart attacks and strokes. It has recently been recognized that inflammation plays an important role in plaque formation.

CD1-restricted T cells are a unique subset of T cells that respond to self and foreign lipid antigens presented by group 1 CD1 (CD1a, -b, -c) and group 2 CD1 (CD1-d) antigen presenting molecules (i.e. macrophages and dendritic cells). Group 2 CD1d molecules are known to associate with invariant NKT cells and induce apoptosis and necrosis in plaques. Numerous studies have shown that NKT cells play a pathogenic role in atherosclerosis using cytokines (IFN-γ, IL-4, IL-17, etc.) that activate other immune cells (e.g. T cells, B cells, and NK cells).

Group 1 CD1 molecules present lipid antigens to a diverse set of T cells with different types of receptors that recognizes lipid antigens specifically, and result in slow and long lasting adaptive-like immune response. Nothing is known about the role of group 1 CD1-restricted T cells in atherosclerosis and hyperlipidemia. This discrepancy is due to lack of an appropriate animal model to conduct such experiments. Mice, commonly used animal model for immunological studies, express only group 2 CD1d, not group 1 CD1 molecules.

This study looks at the unknown role of lipid antigen presenting molecules, specifically CD1b and CD1c subset molecules, on atherosclerotic plaque formation around intima of aortas in new mice models: control LDL receptor knockout mice (**LDLrko**), LDL receptor knockout mice with hCD1 transgene (**hCD1Tg/LDLrko**), LDL receptor and CD1d knockout mice with hCD1 transgene (**hCD1Tg/CD1dko/LDLrko**), and LDL receptor inactivated mice with hCD1and HJ1 transgenes (**hCD1Tg/ HJ1Tg/LDLrko**).

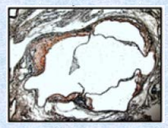
RESULTS

The results of this study indicate that hCD1Tg/CD1dko/ LDLrko group had the highest plaque area with an average of $1.26x10^6$ um^2. Compared to the control mouse strain (LDLrko) with an average plaque area of $9.20x10^5$ um^2, both hCD1Tg/LDLrko and hCD1Tg/ HJ1Tg/LDLrko mice had lower average plaque areas ($8.16x10^5$ and $5.55x10^5$ um^2, respectively).

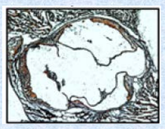
One-way ANOVA and Turkey post-hoc statistical tests were performed. Plaque comparison by sex was also performed

FUTURE RESEARCH & SIGNIFICANCE

Future research with an extended high fat diet period (16-18 weeks) can determine whether HJ1 T cell receptors are activated in hyperlipidemic mice. Clinical uses of this research may include manipulating group 1 CD1-restricted T cells to control heart plaque formation and, possibly, preventing atherosclerosis and heart attacks.



hCD1Tg/HJ1Tg/LDLrko



LDLrko

Figure 1. Oil red O-stained sections of aortic root plaque areas from hCD1Tg/HJ1Tg/LDLrko and LDLrko mice.

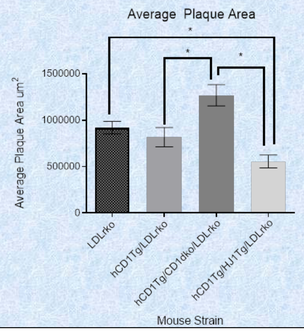


Figure 2: Average plaque areas from each mouse strain are shown, including statistical significance between groups according to Sidak's Multiple Comparison test. *, p<0.05.

	LDLrko	hCD1Tg/ LDLrko	hCD1Tg/ HJ1Tg/ LDLrko	hCD1Tg/ CD1dko/ LDLrko
Average Plaque Area (um^2)	$9.20x10^5$	$8.16x10^5$	$5.55x10^5$	$1.26x10^6$
Standard Deviation	$1.76x10^5$	$2.93x10^5$	$1.71x10^5$	$1.96x10^5$

Table 1. Average plaque areas(um^2) from each group is listed along with the standard deviations.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	$1.08x10^{12}$	3	$3.62x10^{11}$	F (3, 20) = 7.173	P=0.0019
Residual (within columns)	$1.01x10^{12}$	20	$5.05x10^{10}$		
Total	$2.09x10^{12}$	23			

Table 2. One-way ANOVA table outlines sum of squares (SS) between groups and within groups, the degrees of freedom (DF), and mean square (MS). The final results, including the F ratio and P-value, are also stated. P<0.05.

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary
LDLrko vs. hCD1Tg/LDLrko	103438	-235739 to 442616	No	ns
LDLrko vs. hCD1Tg/CD1dko/LDLrko	-348111	-800348 to 104125	No	ns
LDLrko vs. hCD1Tg/HJ1Tg/LDLrko	364844	239.1 to 729449	Yes	*
hCD1Tg/LDLrko vs. hCD1Tg/CD1dko/LDLrko	-451549	-895226 to -7873	Yes	*
hCD1Tg/LDLrko vs. hCD1Tg/HJ1Tg/LDLrko	261406	-92525 to 615337	No	ns
hCD1Tg/CD1dko/LDLrko vs. hCD1Tg/HJ1Tg/LDLrko	712955	249551 to 1176360	Yes	**

Table 3. Sidak's multiple comparisons test was performed to determine the statistical significance between groups. The figure also includes the mean difference, 95% confidence interval of the difference, and statistical significance. *p<0.05; **p<0.001.

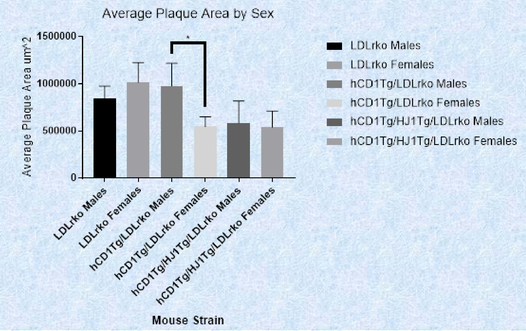


Figure 3. Average plaque area for each sex from the LDLrko, hCD1Tg/LDLrko, and hCD1Tg/HJ1Tg/LDLrko mice. Results of the t-tests significance between sexes are also shown. * p<0.05.

	LDLrko Males	LDLrko Females	hCD1Tg/ LDLrko Males	hCD1Tg/ LDLrko Females	hCD1Tg/ HJ1Tg/ LDLrko Males	hCD1Tg/ HJ1Tg/ LDLrko Females
Average Plaque Area (um^2)	$8.48x10^5$	$1.02x10^6$	$9.78x10^5$	$5.48x10^5$	$5.84x10^5$	$5.41x10^5$
Standard Deviation	$1.28x10^5$	$2.12x10^5$	$2.40x10^5$	$1.01x10^5$	$2.35x10^5$	$1.71x10^5$

Table 4. Average plaque area and standard deviation for each sex in the LDLrko, hCD1Tg/LDLrko, and hCD1Tg/HJ1Tg/LDLrko mice.

Unpaired t test (hCD1Tg/LDLrko Males vs Females)	
P value	0.0282
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.877, df=6

Table 5. T-test results for males and females in only hCD1Tg/LDLrko group showed statistical significance. * p<0.05.

SUMMARY

- Significant unexpected difference in plaque areas between the control mice (LDLrko) and mice with CD1b-restricted T cells (hCD1Tg/HJ1Tg/LDLrko).
- Overexpressing CD1b-restricted T cells (HJ1Tg) decreased plaque count. This refutes the initial hypothesis that CD1b-restricted T cells (HJ1Tg) would increase plaque area.
  - Possibly due to inactivation of HJ1 T cells.
- Significant difference between control (LDLrko) and hCD1Tg/LDLrko mice.
  - hCD1Tg mice do not harbor CD1b and CD1c-restricted T cells.
- hCD1Tg/LDLrko has lower plaque area than hCD1Tg/CD1dko/LDLrko mice
  - CD1d-restricted T cells (i.e. NKT cells) may have an inhibitory effect on CD1c and CD1b-restricted T cell activation or function.
- Unclear whether males usually have a greater plaque formation than females.

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