



LAMPIRAN 1

ETIK

	KEMENTERIAN RISET, TEKNOLOGI, DAN PENDIDIKAN TINGGI UNIVERSITAS BRAWIJAYA FAKULTAS KEDOKTERAN KOMISI ETIK PENELITIAN KESEHATAN Jalan Veteran Malang - 65145, Jawa Timur - Indonesia Telp. (62) (0341) 551611 Ext. 168; 569117; 567192 - Fax. (62) (0341) 564755 http://www.fk.ub.ac.id e-mail : kep.fk@ub.ac.id
	KETERANGAN KELAIKAN ETIK ("ETHICAL CLEARANCE") No. 336 / EC / KEPK / 09 / 2016
KOMISI ETIK PENELITIAN KESEHATAN FAKULTAS KEDOKTERAN UNIVERSITAS BRAWIJAYA, SETELAH MEMPELAJARI DENGAN SEKSAMA RANCANGAN PENELITIAN YANG DIUSULKAN, DENGAN INI MENYATAKAN BAHWA PENELITIAN DENGAN	
JUDUL	: Pengembangan Metode Elicit Dose Antigen Specific immunotherapy Menggunakan Self Antigen dsDNA sebagai Terapi Baru Perbaikan Regulasi Sistem Imun Pada Lupus Eritematosus Sistemik.
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UNIT / LEMBAGA	: Fakultas Kedokteran – Universitas Brawijaya
TEMPAT PENELITIAN	: Laboratorium Sentral Biomedik dan Laboratorium Farmakologi Fakultas Kedokteran Universitas Brawijaya
DINYATAKAN LAIK ETIK.	
Malang, 08 SEP 2016  Ketua, Komis Etik Penelitian Kesehatan Prof. Dr. dr. Moch. Istiadjid ES, SpS, SpBS (K), M.Hum NIP. 194605161971111 1 001	
Catatan : Keterangan Laik Etik Ini Berlaku 1 (Satu) Tahun Sejak Tanggal Dikeluarkan Pada Akhir Penelitian. Laporan Pelaksanaan Penelitian Harus Diserahkan Kepada KEPK-FKUB Dalam Bentuk	

LAMPIRAN 2

ANALISA STATISTIK

- Lampiran Analisa Statistik Manifestasi Klinis (Berat Badan (BB)) Mencit Normal Dan Mencit Lupus Induksi Pristane Secara Berkala

a. b. Uji Deskriptif kadar Berat Badan mencit lupus induksi *Pristane*

	Kelompok	N	Mean	Std. Deviation
Adaptasi	Mencit Normal	5	21.0000	2.00000
	Mencit Lupus	20	20.6250	1.97402
	Induksi <i>Pristane</i>			
Sebelum Injeksi <i>Pristane</i>	Mencit Normal	5	26.1667	2.71416
	Mencit Lupus	20	24.9583	3.59322
	Induksi <i>Pristane</i>			
4 Minggu Setelah Injeksi <i>Pristane</i>	Mencit Normal	5	31.3333	3.98330
	Mencit Lupus	20	31.4583	4.13867
	Induksi <i>Pristane</i>			
12 Minggu Setelah Injeksi <i>Pristane</i>	Mencit Normal	5	36.8333	3.37145
	Mencit Lupus	20	30.4583	4.15963
	Induksi <i>Pristane</i>			

c. Tests of Normality kadar Berat Badan mencit lupus induksi *Pristane*

	Kelompok_	Shapiro-Wilk	
		Statistic	Sig.
Adaptasi	Mencit Normal	.976	.933
	Mencit PIL	.890	.013
Sebelum_Injeksi	Mencit Normal	.859	.184
	Mencit PIL	.859	.003
Empatminggusetelahinjeksi	Mencit Normal	.944	.689
	Mencit PIL	.963	.511
Duabelasminggusetelahinjeksi	Mencit Normal	.976	.931
	Mencit PIL	.957	.381

d. Uji Homogenitas Berat Badan mencit normal dan mencit lupus induksi *pristane* secara berkala

		Levene's Test for Equality of Variances	
		F	Sig.
Adaptasi	Equal variances assumed	.175	.679
	Equal variances not assumed		
Sebelum Injeksi <i>Pristane</i>	Equal variances assumed	.064	.802
	Equal variances not assumed		
4 Minggu Setelah Injeksi <i>Pristane</i>	Equal variances assumed	.072	.791
	Equal variances not assumed		
12 Minggu Setelah Injeksi <i>Pristane</i>	Equal variances assumed	.612	.441
	Equal variances not assumed		

• Lampiran Analisa Statistik Manifestasi Serologis (Kadar Anti-dsDNA) Mencit Lupus Induksi *Pristane*

a. Uji Deskriptif kadar anti-dsDNA mencit lupus induksi *Pristane*

	Group	N	Mean	Std. Deviation
Anti_dsDNA	K-	5	7.8509	5.00367
	K+	5	80.3581	47.29211

b. Uji Normalitas kadar anti-dsDNA mencit lupus induksi *Pristane*

	Group	Shapiro-Wilk	
		Statistic	Sig.
Anti_dsDNA	K-	.848	.218
	K+	.923	.553

c. Uji Homogenitas kadar anti-dsDNA mencit lupus induksi *Pristane*.

		Levene's Test for Equality of Variances	
		F	Sig.
Anti_dsDNA	Equal variances assumed	31.582	.001
	Equal variances not assumed		

d. Uji Mann-Whitney kadar anti-dsDNA mencit lupus induksi Pristane

	Anti_dsDNA
Mann-Whitney U	.000
Wilcoxon W	10.000
Z	-2.309
Asymp. Sig. (2-tailed)	.021
Exact Sig. [2*(1-tailed Sig.)]	.029

• Lampiran Analisa Statistik Pemberian Terapi EDI dsDNA Terhadap Manifestasi Klinis (Berat Badan (BB)) Pada Mencit Lupus Induksi *Pristane*

a. Terapi EDI dsDNA terhadap Berat Badan (BB) pada mencit lupus Induksi *Pristane*

	N	Mean	Std. Deviation
K (-)	5	37.6667	4.50185
K (+)	5	29.0000	2.75681
A	5	33.1667	2.31661
B	5	29.0000	3.22490
C	5	32.5000	3.27109
Total	25	32.2667	4.47162

b. Uji Normality terapi EDI dsDNA terhadap Berat Badan (BB) pada mencit lupus Induksi *Pristane*

Kelompok	Shapiro-Wilk	
	Statistic	Sig.
K (-)	.975	.923
K (+)	.971	.899
BB A	.823	.094
B	.804	.064
C	.894	.340

c. Uji Homogeneity Terapi EDI dsDNA terhadap Berat Badan (BB) pada mencit lupus Induksi *Pristane*

Levene Statistic	df1	df2	Sig.
.693	4	25	.604

d. Uji Post Hoc Terapi EDI dsDNA Terhadap Berat Badan (BB) Pada Mencit Lupus Induksi *Pristane*

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Sig.
K (-)	K (+)	8.66667 [*]	.000
	A	4.50000 [*]	.026
	B	8.66667 [*]	.000
	C	5.16667 [*]	.012
K (+)	K (-)	-8.66667 [*]	.000
	A	-4.16667 [*]	.038
	B	.00000	1.000
	C	-3.50000	.078
A	K (-)	-4.50000 [*]	.026
	K (+)	4.16667 [*]	.038
	B	4.16667 [*]	.038
	C	.66667	.729
B	K (-)	-8.66667 [*]	.000
	K (+)	.00000	1.000
	A	-4.16667 [*]	.038
	C	-3.50000	.078
C	K (-)	-5.16667 [*]	.012
	K (+)	3.50000	.078
	A	-.66667	.729
	B	3.50000	.078

- **Lampiran Analisa Statistik Pemberian Terapi EDI dsDNA Terhadap Jumlah Sel Th1 Pada Mencit Lupus Induksi *Pristane***

a. Uji Normalitas Jumlah Th1

One-Sample Kolmogorov-Smirnov Test		
		Jumlah Th1
Normal Parameters ^a	Mean	3.1642
	Std. Deviation	1.01226
Most Extreme Differences	Absolute	.168
	Positive	.168
	Negative	-.164
Kolmogorov-Smirnov Z		.856
Asymp. Sig. (2-tailed)		.457
N		25

b. Uji Homogenitas Jumlah Th1

Levene Statistic	df1	df2	Sig.
2.371	4	21	.085

c. Uji ANOVA Jumlah Th1

Jumlah Th1	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.163	4	.291	.716	.591
Within Groups	8.529	21	.406		
Total	9.692	25			

d. Lampiran analisa statistik Pemberian terapi EDI dsDNA terhadap kadar interferon gamma (IFN- γ) pada mencit lupus induksi *pristane*.

a. Uji Normalitas Kadar Interferon Gamma (IFN- γ)

		Kadar Interferon Gamma
Normal Parameters ^a	Mean	10.1778
	Std. Deviation	3.60695
Most Extreme Differences	Absolute	.170
	Positive	.170
	Negative	-.097
Kolmogorov-Smirnov Z		.721
Asymp. Sig. (2-tailed)		.677
N		25

b. Uji Homogenitas Kadar Interferon Gamma (IFN- γ)

Levene Statistic	df1	df2	Sig.
10.741	4	13	.000

c. Uji Kruskal Wallis Kadar Interferon Gamma (IFN- γ)

		Kadar Interferon Gamma (IFN- γ)
Chi-Square		11.658
Df		4
Asymp. Sig.		.020
N		25

d. Mann Whitney Kadar Interferon Gamma (IFN- γ)

		P
K-	K+	0.021
K-	A	1.000
K-	B	0.773
K-	C	0.064
K+	A	0.021
K+	B	0.021
K+	C	0.165
A	B	0.773
A	C	0.064
B	C	0.064

e. Lampiran Analisa Statistik Pemberian Terapi EDI dsDNA Terhadap Kadar Interferon Gamma (IFN- γ) Pada Mencit Lupus Induksi *Pristane*

Correlations

			Dosis terapi EDI dsDNA	Kadar (IFN- γ)
Spearman's rho	Dosis terapi	Correlation Coefficient	1.000	.467
	EDI dsDNA	Sig. (2-tailed)	.	.173
		N	25	25
	Kadar (IFN- γ)	Correlation Coefficient	.467	1.000
		Sig. (2-tailed)	.173	.
		N	25	25

f. Lampiran Analisa Statistik Pemberian Terapi EDI dsDNA Terhadap Jumlah Sel Th1 Pada Mencit Lupus Induksi *Pristane*

Correlations

			Dosis terapi EDI dsDNA	Jumlah sel Th1
Spearman's rho	Dosis terapi	Correlation Coefficient	1.000	.298
	EDI dsDNA	Sig. (2-tailed)	.	.402
		N	25	25
	Jumlah sel Th1	Correlation Coefficient	.298	1.000
		Sig. (2-tailed)	.402	.
		N	25	25

LAMPIRAN 3
DUMMY TABLE

1. Manifestasi Klinis Dan Manifestasi Serologis Mencit Lupus Induksi

Pristane (Dummy Table)

	BB				Bulu Rontok		Aktivitas		Anti-dsDNA
	Pre		Post		Pre	Post	Pre	Post	
	Adaptasi	Sebelum Injeksi Pristane	4 Minggu Setelah Injeksi Pristane	12 Minggu Setelah Injeksi Pristane					
Mancit Normal	21.0 ± 2.00	26.2 ± 2.71	31.3 ± 3.98	36.8 ± 3.37	Tidak ada	Tidak Ada	Tidak ada penurunan	Tidak ada penurunan	7.9 ± 5.0
Mencit PIL	20.6 ± 1.97	25.0 ± 3.59	31.5 ± 4.14	30.5 ± 4.16	Tidak ada	Ada	Tidak ada penurunan aktifitas	Ada penurunan aktifitas	80.4 ± 47.3

2. Efek Terapi EDI dsDNA Terhadap Mencit Lupus Induksi *Pristane*

(Dummy Table)

	BB (g)	Bulu Rontok		Penurunan Aktivitas	
		Pre	Post	Pre	Post
K (-) n=5	37.7 ± 4.5	0/5	0/5	0%	0%
K (+) n=5	29.0 ± 2.8	5/5	5/5	Menurun (100%)	Tetap (100%)
A n=5	33.2 ± 2.3	5/5	5/5	Menurun (100%)	Tetap (100%)
B n=5	29.0 ± 3.2	5/5	5/5	Menurun (100%)	Tetap (100%)
C n=5	32.5 ± 3.3	5/5	5/5	Menurun (100%)	Tetap (100%)

LAMPIRAN 4

DATA AKTIVITAS MENCIT LUPUS INDUKSI *PRISTANE*

Perlakuan	penurunan aktivitas			
	saat adaptasi	saat injeksi BMC	4 minggu setelah injeksi BMC	12 minggu setelah injeksi BMC
Mencit Normal				
1	300	310	610	203
2	310	320	630	210
3	320	330	650	217
4	330	340	670	223
5	340	350	690	230
6	350	360	710	237
Mencit Lupus Induksi <i>Pristane</i>				
1	360	370	730	243
2	370	380	750	250
3	380	390	770	257
4	390	400	790	263
5	400	410	810	270
6	410	420	830	277
7	420	430	850	283
8	430	440	870	290
9	440	450	890	297
10	450	460	910	303
11	460	470	930	310
12	470	480	950	317
13	480	490	970	323
14	490	500	990	330
15	500	510	1010	337
16	510	520	1030	343
17	520	530	1050	350
18	530	540	1070	357
19	540	550	1090	363
20	550	560	1110	370
21	560	570	1130	377
22	570	580	1150	383
23	580	590	1170	390
24	590	600	1190	397

	Pre EDI ds DNA	Post EDI dsDNA
K+		
	243	292
	250	300
	257	308
	263	316
	270	324
	277	332
A		
	283	340
	290	348
	297	356
	303	364
	310	372
	317	380
B		
	323	388
	330	396
	337	404
	343	412
	350	420
	357	428
C		
	363	436
	370	444
	377	452
	383	460
	390	468
	397	476

LAMPIRAN 5

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Title	Desensitization of Self-Antigen dsDNA Inhibits B and T cell Functions by Modulating T-Reg cell as Regulator Immune System in Pristane-induced Lupus Mice Model
Running Title	SLE, dsDNA, escalating dose, T-reg
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Section	Basic Immunology
Corresponding Author	Prof. Kusworini Handono (Brawijaya University)
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Abstract	<p>Background: SLE is an autoimmune disease. Immunosuppressant and steroid therapy have not shown satisfactory results. Another method of therapy that is now also being developed is vaccines and escalating dose immunotherapy using self-antigen.</p> <p>Objective: The aim of this study was to develop a novel therapeutic method for improving immune system regulation in SLE using self-antigen dsDNA in pristane-induced lupus mice model.</p> <p>Methods: 25 female BALB/c mice were divided into 2 groups: 20 mice received a single i.p. injection of 0.5 cc pristane and 5 mice as healthy controls. 8 weeks after injection, 15 pristane-induced lupus mice were divided into three groups: 0,005 µg, 0,05 µg, and 0,5 µg. The doses would increase ten times every week. dsDNA were complexed with PEI. A total of 25 BALB/c mice were analyzed for autoantibodies dsDNA and ANA, proinflammatory cytokines IL-17, and TGF-β from serum using ELISA and T-Reg, Th17, B cell proliferation from spleen using flow cytometry.</p> <p>Results: Escalating dose antigen specific immunotherapy with dsDNA decreased ANA levels (30.42 vs 1.6 p=0.02) significantly, decreased anti-dsDNA (63.4 vs 5.6 p=0.02), decreased dendritic cell maturation (2.43 vs 0.75 p=0.02) and not significantly decreases Th17 cells (5,62 vs 3,62 p=0,18) but the result tend to get lower. Increased T-reg proliferation (10.57 vs 18.38 p=0.00) and level of TGF-β (261.02 vs 732 p=0.03) significantly compare to control positive.</p> <p>Conclusion: Desensitization using self-antigen dsDNA was able to modulate T-Reg and inhibit B and T cell functions in lupus mice model.</p> <p>Keywords: SLE, dsDNA, escalating dose, tolerance, T-regulator</p>