Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products, Russian Academy of Sciences

Laboratory of modeling of immunobiological processes with experimental clinic of common marmosets

Nonhuman primate models for preclinical testing of cancer vaccines

Ilya Gordeychuk

Workshop “IMMUNOTHERAPY OF CANCER”

project “New approach to active immunotherapy of hepatitis c related cancer”LZP-2018/2-0308

Riga Stradins University, Riga, Latvia

13 June 2019

Common marmoset breeding facility

Common marmosets (Callithrix jacchus)

Sharp increase in the use of laboratory marmosets

Small and social, marmosets offer advantages for researchers.

ANIMAL RESEARCH

U.S. labs clamor for marmosets

Shortage develops as new transgenic models for neurological diseases stoke interest

Science, 26 October 2018

Advantages and disadvantages of marmosets in biomedical research

Advantage	
Proximity to humans	Genetics, (neuro) anatomy, immunology, physiology, microbiology.
Biology	Relatively small (300–350 grams) compared with other nonhuman primates (e.g., macaque species), high reproductive efficiency in captivity, lower caging and feeding costs compared with macaques, socially housed.
Conventional housing	Exposure of immune-shaping pathogens from the external milieu (e.g., gut microbiotica and environment) and from the internal milieu (e.g., opportunistic infection with herpes viruses such as the marmoset counterparts of Epstein-Barr virus and cytomegalovirus).
Outbred nature	Comparable genetic heterogeneity to the human population. Wild populations are not endangered.
Cross-reactivity	Biological therapeutics developed for human diseases e.g., monoclonal antibodies and cytokines, can be assessed for preclinical evaluation of efficacy, safety, and mechanism of action.
Bone-marrow chimerism	Twins or triplets are immunologically highly similar, and hence can be used in pairs for therapeutics studies. Twin siblings are mutually allotolerant, enabling adoptive transfer of cells between siblings.
Drug development	Cheaper due to small size, 10- to 20-fold less of an experimental drug is needed compared to macaques.
Disadvantage	
Costs	Relatively high compared with rodents or other non-rodent species.
Cross-reactivity	Limited availability of diagnostic reagents such as monoclonal antibodies for flow cytometry and immunohistochemistry.
Ethical	Are closer to humans compared with rodents, limited possibilities for experimental manipulations (e.g., transgenic experiments).
Size	Small size, difficult or impossible to perform certain procedures or techniques (e.g., MRI of spinal cord), small volume of blood or organs (e.g., lymph nodes) can be obtained to perform *ex vivo* experiments.

Jagessar et al. *Exp. Anim. 2013*

Antibody clones for flow cytometry

NHP REAGENT RESOURCE — MassBiologics UMASS Medical School, Medicine for Better Lives

Vendor/Clone	African Green (*Chlorocebus pygerythrus*)	Capuchin Monkey (*Cebus capucinus*)	Chimpanzee (*Pan troglodytes*)	Common Marmoset (*Callitrix jacchus*)	Cotton-topped Tamarin (*Sanguinus oedipus*)	Cynomolgus Monkey (*Macaca fascicularis*)	Hamadryas Baboon (*Papio hamadryas*)	Olive Baboon (*Papio anubis*)	Owl Monkey (*Aotus trivirgatus*)	Pigtailed Macaque (*Macaca nemestrina*)	Rhesus (*Macaca mulatta*)	Sooty Mangabey (*Cercocebus torquatus*)	Squirrel Monkey (*Salmini sciureus*)
BD Biosciences SP34	–	–	✓	✓	–	✓	✓	✓	–	✓	✓	✓	✓
Invitrogen FN18	–	–	✓	–	–	✓	–	✓	–	✓	✓	–	✗
Miltenyi 10D12	✓	–	–	✗	✗	✓	–	✓	–	✓	✓	–	–
U-CyTech FN18	–	–	✗	✗	✗	–	–	✓	–	✓	✓	–	–
Mabtech CD3-1	–	–	–	–	–	✓	–	–	–	–	✓	–	–
AbD Serotec/Bio-Rad FN18	–	–	–	–	–	✓	–	–	–	–	✓	–	–
Sony Biotech SK7	–	–	✓	–	–	✗	–	–	–	–	✓	–	–
AbD Serotec/Bio-Rad CD3-12	–	–	–	–	–	–	–	–	–	–	✓	–	–
BioLegend HIT3a	–	–	✓	–	–	–	–	–	–	–	–	–	–
BioLegend SK7	–	–	✓	–	–	–	–	–	–	–	–	–	–
AbD Serotec/Bio-Rad UCHT1	–	–	✓	–	–	–	–	–	–	–	–	–	–
BioLegend UCHT1	–	–	✓	–	–	✗	–	–	–	–	✗	–	–
Sony Biotech UCHT1	–	–	✓	–	–	✗	–	–	–	✗	–	–	✗
BD Biosciences SK-7	–	–	✓	–	–	–	✗	–	–	✗	✗	–	✗
BD Biosciences UCHT1	–	–	✓	–	–	✗	–	–	–	✗	✗	–	✗

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An Extensive Monoclonal Antibody Panel for the Phenotyping of Leukocyte Subsets in the Common Marmoset and the Cotton-Top Tamarin

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New World monkeys are va study human diseases. To d of cells involved in immune cytometry to screen a larg monoclonal antibodies (m with cells of the common m top tamarin. Certain antiger are well conserved. Howeve showed a clear discrepanc terns in both species, indica ferences on the epitope lev lution. Epstein-Barr virus-t were shown to be a valu B-cell–specific reagents. In

Comprehensive panel of cross-reacting monoclonal antibodies for analysis of different immune cells and their distribution in the common marmoset (*Callithrix jacchus*)

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Keywords
flow cytometry – immunophenotyping – innate and adaptive immunity – New World monkeys

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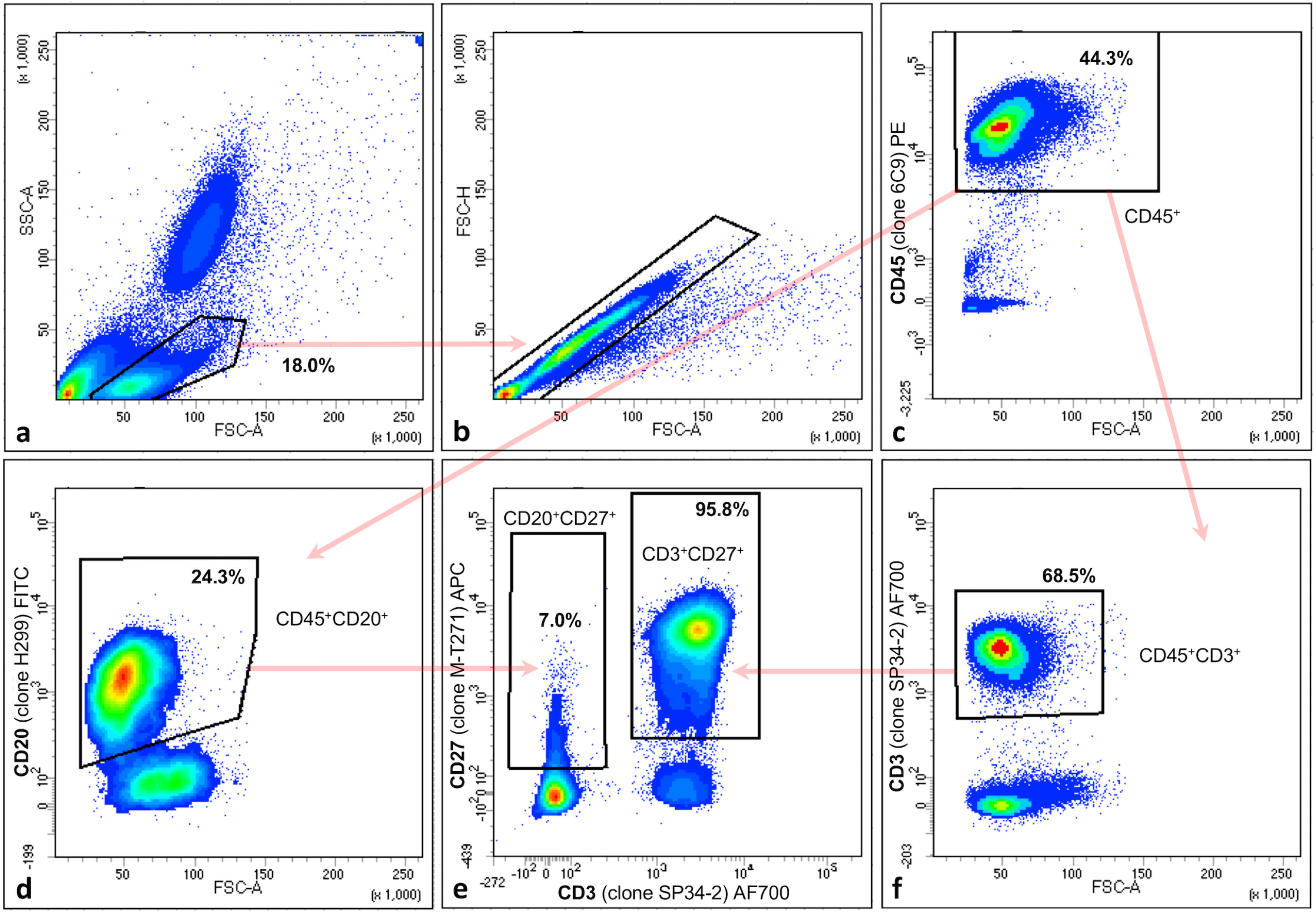
Abstract

Background Common marmosets are extensively used in immunological and pharmacological research, and the usage of methods such as flow cytometry gain increasing importance.
Methods Using multicolor flow cytometry cross-reactivity of monoclonal antibodies with cells of common marmosets was analyzed. Furthermore, frequencies of immune cells and immunological parameters were assessed in healthy common marmosets.
Results A total of 97 clones of monoclonal antibodies raised against CD markers, chemokine receptors, and miscellaneous markers were tested. Additionally, baseline frequencies of different innate and adaptive immune cells as well as certain parameters, such as activation and memory T-cell and B-cell distribution, are provided.
Conclusion Our study gives an extended overview of cross-reactive antibodies for flow cytometric analysis of immune cells as well as baseline values for different immune parameters in healthy common marmosets.

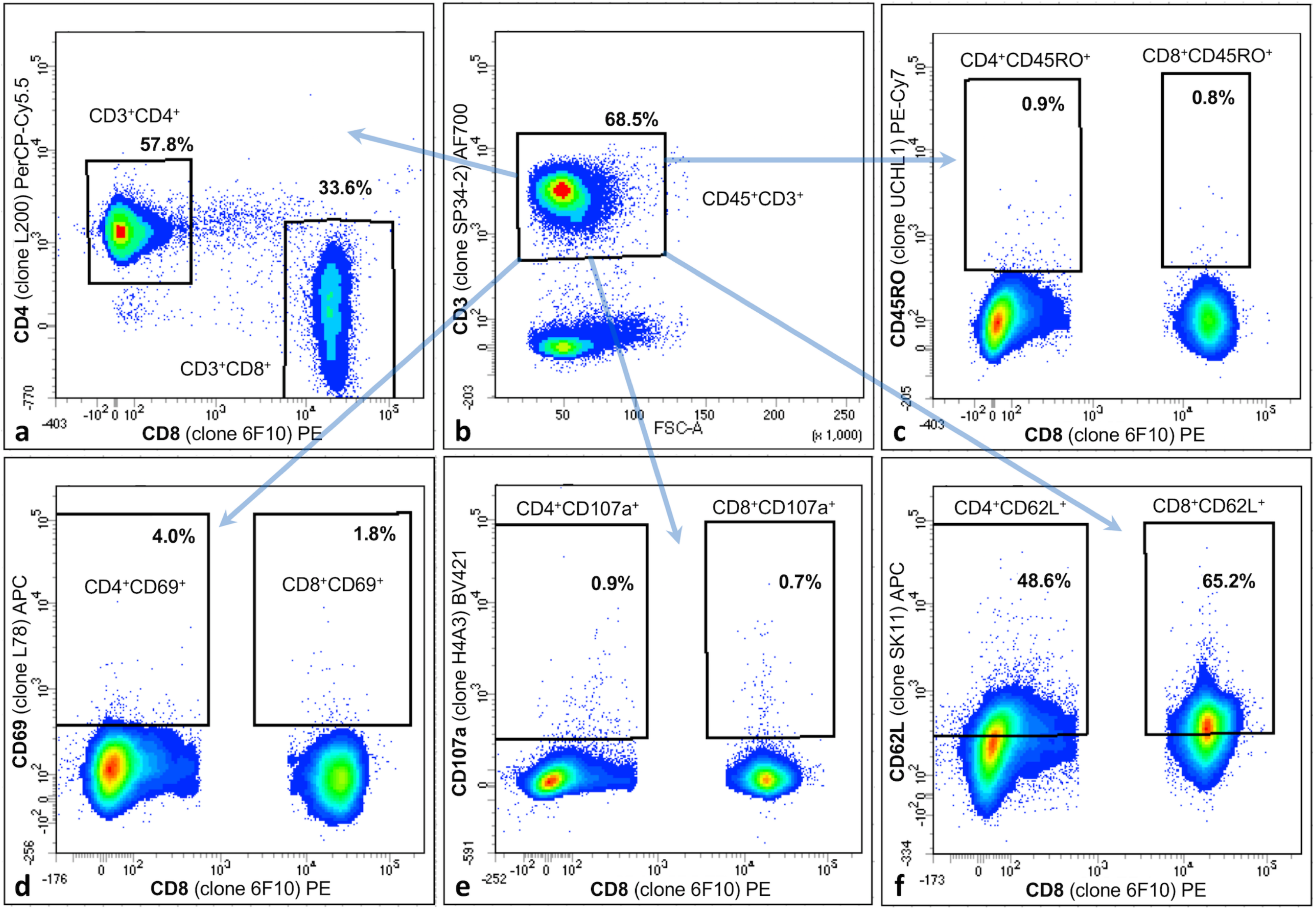
Antibody clones for flow cytometry

Marker	Antibody clone	Fluorochrome	Reactivity	Manufacturer
CD45	6C9	PE	marmoset	BioLegend
CD3	SP34-2	Alexa Fluor 700	human	BD
CD20	H299	FITC	human	Beckman Coulter
CD4	L200	PerCP-Cy5.5	human	BD
CD8	6F10	PE	marmoset	BioLegend
CD69	L78	APC	human	BD
CD62L	SK11	BV421	human	BD
CD45RO	UCHL1	PE/Cy7	human	BioLegend
CD107A	H4A3	BV421	human	BD
CD27	M-T271	APC	human	BioLegend

Gating strategy and identification of the populations of T-and B-cells



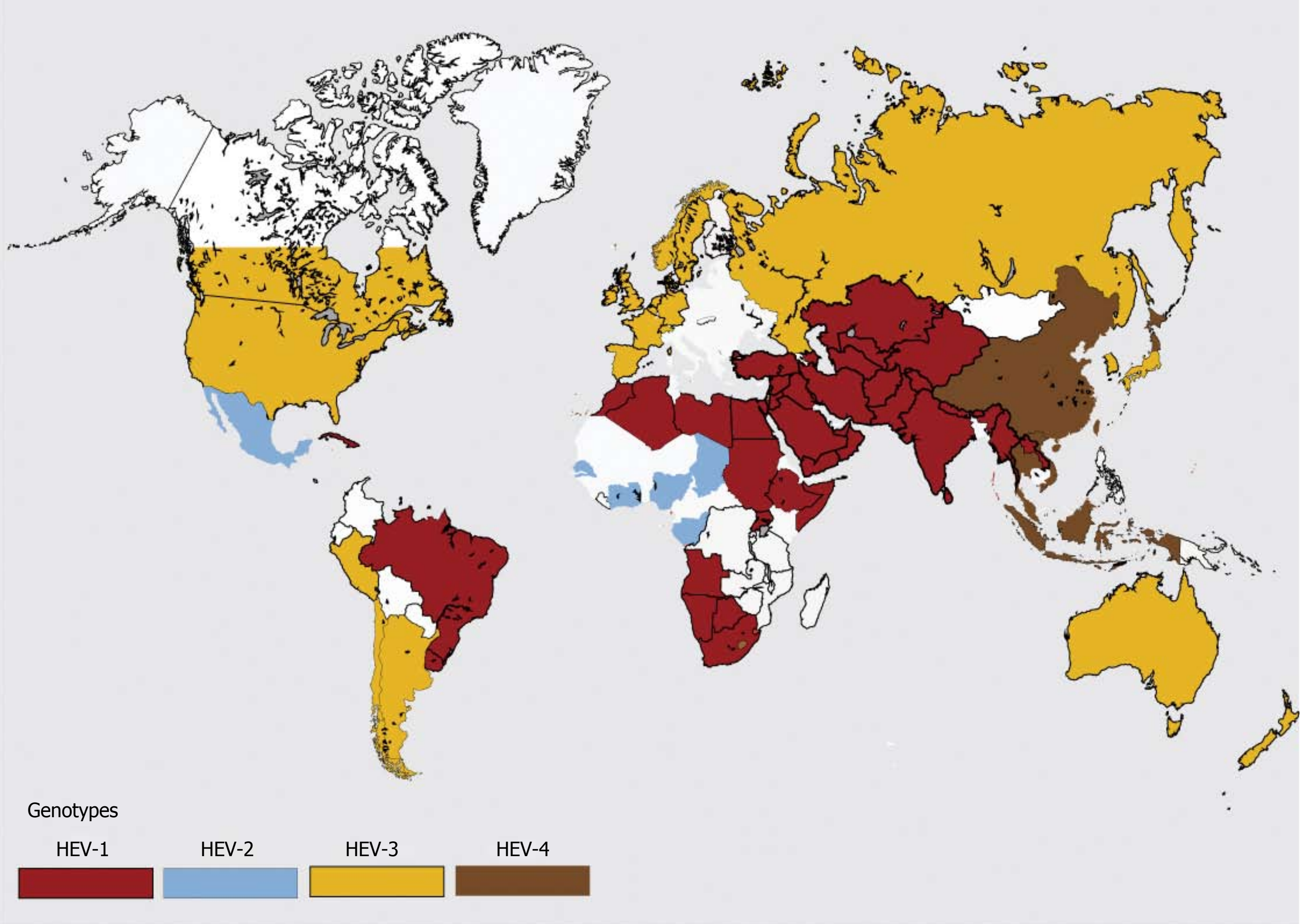
Identification of T-cell subpopulations



Proportions of reactive peripheral blood cells of naïve CMs

Parameter	Marmoset ID, parameter %										Total, M±σ, %
	Female						Male				
	2996	2998	0519	3016	2997	M±σ,	2994	4540	4520	M±σ,	
Age, months	29	29	23	48	25	30.8±10.0	30.0	25.0	25.0	26.7±2.9	29.3±8.0
*CD45$^+$	67.5	64.5	62.3	43.5	43.2	56.2±11.9	42.1	44.3	66.6	51.0±13.6	54.3±11.8
CD45$^+$CD3$^-$CD20$^+$	28.7	32.4	17.7	17.5	20.4	23.3±6.8	22.3	24.3	18.4	21.7±3.0	22.7±5.5
CD45$^+$CD20$^+$CD27$^+$	8.3	11.8	5.9	17	7.9	10.2±4.4	8.9	7.0	4.7	6.9±2.1	8.9±3.9
CD45$^+$CD3$^+$CD20$^-$	62.4	57.6	69.6	74.7	64.4	65.7±6.6	66.5	68.5	76.9	70.6±5.5	67.6±6.3
CD45$^+$CD3$^+$CD27$^+$	93.9	93.2	96.2	98.4	93.2	95.0±2.3	91.8	95.8	94.6	94.1±2.1	94.6±2.1
CD3$^+$CD4$^-$CD8$^+$	39.2	32.7	34.4	40	32.9	35.8±3.5	33.2	33.6	28.5	31.8±2.8	34.3±3.7
CD3$^+$CD8$^+$CD62L$^+$	72.7	81.2	89.3	86.7	51.8	76.3±15.1	76.4	65.2	72.0	71.2±5.6	74.4±12.1
CD3$^+$CD8$^+$CD69$^+$	0.9	1.1	1.6	1.9	0.3	1.2±0.6	1.2	1.8	1.0	1.3±0.4	1.2±0.5
CD3$^+$CD8$^+$CD45RO$^+$	2	2.4	1.8	1.8	0.8	1.8±0.6	2.0	0.8	0.7	1.2±0.7	1.8±0.7
CD3$^+$CD8$^+$CD107a$^+$	0.9	0.5	0.8	0.5	0	0.5±0.4	0.2	0.7	0.2	0.4±0.3	0.5±0.3
CD3$^+$CD4$^+$CD8$^-$	49.9	57.7	51.2	49.7	57.8	53.3±4.1	55.5	57.8	66.1	59.8±5.6	55.7±5.5
CD3$^+$CD4$^+$CD62L$^+$	47.3	56	73.8	66	43	57.2±12.8	49.1	48.6	47.8	48.5±0.7	54.0±10.7
CD3$^+$CD4$^+$CD69$^+$	1.1	2.3	3.8	4.2	1.7	2.6±1.3	2.0	4.0	2.7	2.9±1.0	2.7±1.2
CD3$^+$CD4$^+$CD45RO$^+$	2	1.7	2.3	2.4	1.1	1.9±0.5**	1.3	0.9	1.0	1.1±0.2**	1.6±0.6
CD3$^+$CD4$^+$CD107a$^+$	1.2	0.6	1.5	0.9	0.2	0.9±0.5	0.2	0.9	0.4	0.5±0.4	0.7±0.5

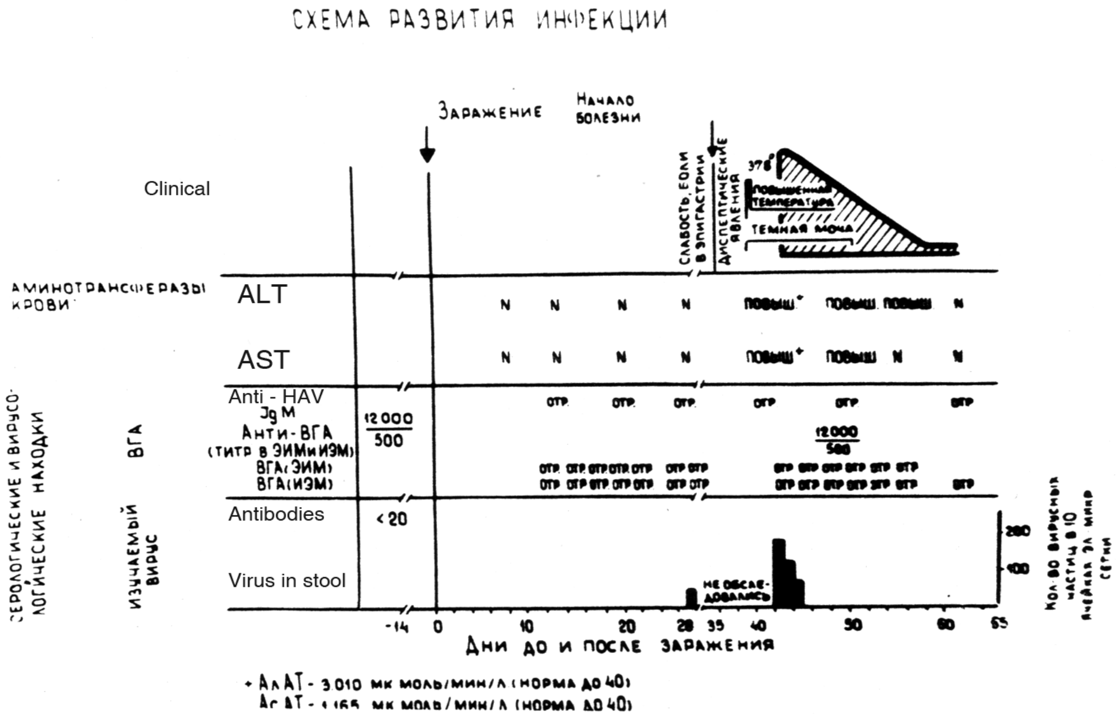
Hepatitis E virus worldwide genotype distribution



MS Khuroo et al. World J Gastroenterol 2016

Hepatitis E virus discovery in a self-infection experiment in 1981

Mikhail S Balayan
(1933–2000)



Non-human primates susceptible to HEV

Rhesus monkeys (Macaca mulatta)

Cynomolgus monkeys
(Macaca fascicularis)

Common marmosets (Callithrix jacchus)

The current state of the development of HEV vaccine

- HEV propagates poorly in cell culture – no perspectives for inactivated or attenuated vaccine
- HEV capsid protein is a main target for neutralizing antibodies and is a backbone for all HEV prototype vaccines
- The native HEV capsid protein contains conformational epitopes for neutralizing antibodies (C-terminus of the protein, aa 459–606) exposed on the surface of the virion
- At least 11 experimental recombinant vaccines were tested for efficacy in challenge experiments, only two vaccines were brought to the stage of clinical trials in humans
- Only one vaccine (Hecolin, HEV 239) was licensed in China for use in humans
- rHEV vaccine (56 kDA) developed by GlaxoSmithKline did not become commercially available

Common marmoset (Callithrix jacchus) immunization design

Animal ID	Immunization 1	Immunization 2	Immunization 3	Booster immunization	Challenge
	Week 0	Week 3	Week 6	Week 17	Week 25
M1	20 µg/alum	20 µg/alum	20 µg/alum	20 µg/alum	HEV Gt1
M2	20 µg/alum	20 µg/alum	20 µg/alum	20 µg/alum	HEV Gt1
M3	20 µg/alum	20 µg/alum	20 µg/alum	20 µg/alum	HEV Gt3
M4	20 µg/alum	20 µg/alum	20 µg/alum	20 µg/alum	HEV Gt3
M5	alum	alum	alum	alum	HEV Gt1
M6	alum	alum	alum	alum	HEV Gt3

Challenge on week 8 after booster immunization

- Gt1 – human HEV, Gt3 – swine HEV
- Intravenous inoculation with HEV Gt1 or Gt3, 10^6 copies/ml
- Sterile 10% fecal suspension
- Inoculum volume: 1 ml (10^6 HEV RNA copies)
- HEV RNA in feces testing: daily (till week 9 post infection)
- Serum HEV RNA and anti-HEV testing: once weekly till week 9 post infection

HEV RNA detection in feces

Week post infection	Animal ID (vaccine/challenge)					
	M1 (vaccine/ HEV Gt1)	M2 (vaccine/ HEV Gt1)	M1 (vaccine/ HEV Gt3)	M1 (vaccine/ HEV Gt3)	M1 (placebo/ HEV Gt1)	M1 (placebo/ HEV Gt3)
0	neg	neg	neg	neg	neg	neg
1	neg	neg	neg	neg	neg	neg
2	neg	neg	neg	neg	pos	pos
3	neg	neg	neg	neg	pos	pos
4	neg	neg	neg	neg	pos	neg
5	neg	neg	neg	neg	pos	neg
6	neg	neg	neg	neg	pos	neg
7	neg	neg	neg	neg	neg	neg
8	neg	neg	neg	neg	neg	neg
9	neg	neg	neg	neg	neg	neg

Conclusion

With the newly developed methods marmosets have become one of the most widely used and effective models for fundamental research and preclinical testing of immunobiological drugs

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Thank you for your attention!