Immunophenotyping cells of the immune system of birds

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Introduction

Immunohistochemistry is an histopathological process based in the use of special designed antibodies for antigen specifically binding. This technique is widely used as a diagnostic tool in birds in general to bind some specific antigens, specially virical. However, the immune system cell of birds are not so easy to stain with this technique; in fact, only the CD3 antibody in front CD3 T cells is accepted as the only useful antibody in this task.

Objectives

To test new antibodies towards immune system cells of birds (chicken specially), whose combined with specific immunohistochemistry techniques, would become an useful tool for study and diagnosis.

Materials and methods

- Formalin-fixed paraffin-embedded (FFPE) tissue sections from young chicken including: bursa of Fabricius, thymus, liver, kidney, spleen and heart.
- Antibodies:
  - **Anti-CD4** Mouse antichicken CD4 Isotype Mouse (BALB/C) IgG1k, 0,5 mg/mL.
  - **Anti-CD8α** Mouse antichicken CD8a Isotype Mouse (BALB/C) IgG1k, 0,5 mg/mL.
  - **Anti-CD79α [HM47/A9]** Mouse monoclonal [HM47/A9] Isotype IGG1, 0,2 mg/mL.
  - **Lyzozyme EC.3.2.1.17** Polyclonal rabbit anti-human.

Results

The multiple results are presented in the table below (see Table 1), according the used antibody and its dilution and incubation temperature along the antigen retrieval method in every single one organ. The positives tests provided by lysozyme are visible in the images next to.

Table 1. Results are reported by each organ in a coloured way: absence of staining, non specific staining or specific staining

Non staining | Non specific staining | Specific staining

Primary antibody	Dilution	Incubation T°	Ag retrieval method	Bursa of Fabricius	Thymus	Liver	Kidney	Spleen	Heart
Anti - CD4	1:10	4°C	0,1% Tripsyn						
			Proteinase K						
			EDTA						
			Citrate buffer						
	1:25	4°C	Proteinase K						
			EDTA						
			Citrate buffer						
	1:50	4°C	No enzymatic treatment						
			Proteinase K						
			EDTA						
			Citrate buffer						
Anti - CD8α	1:10	4°C	0,1% Tripsyn						
			Proteinase K						
			EDTA						
			Citrate buffer						
	1:25	4°C	Proteinase K						
			EDTA						
			Citrate buffer						
	1:50	4°C	No enzymatic treatment						
			Proteinase K						
			EDTA						
			Citrate buffer						
Anti - CD79α	1:25	4°C	EDTA						
		Environmental T°	EDTA						
			EDTA						
	1:50	4°C	Proteinase K						
			EDTA						
			Citrate buffer						
		Environmental T°	EDTA						
			EDTA						
	1:100	Environmental T°	EDTA						
Lysozyme	1:100	4°C	0,1% Tripsyn						
	1:250	4°C	0,1% Tripsyn						
	1:500	4°C	0,1% Tripsyn						

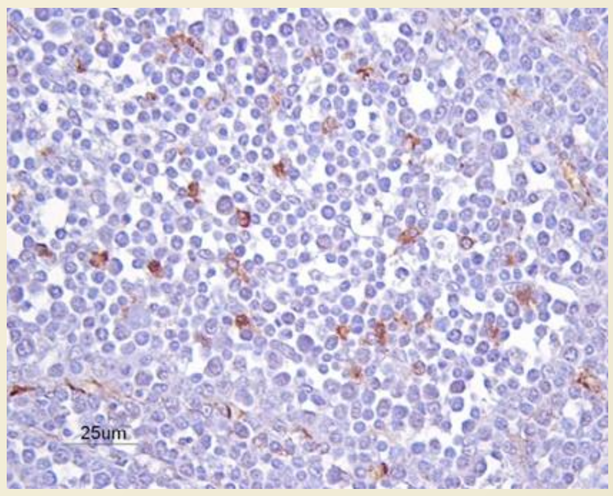

Image 1.
Bursa of Fabricius, chicken. Follicular medullar zone. Positive staining can be seen in some cells, which are presented in brown colour. The staining is localized in the cytoplasm and has a granular appearance.

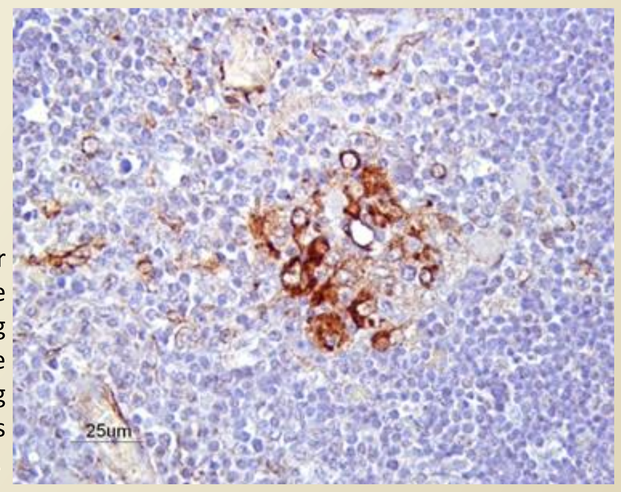

Image 2.
Thymus, chicken. Follicular medullar zone. As the image upon, some positive staining can be appreciate in the cytoplasm of polygonal, big spherical nucleated cells compatible with macrophages.

Discussion & Conclusions

The results obtained from the different tests are very poor. Neither anti-CD4, anti-C84 or anti-CD79 provided any positive result in any of the tested tissues. Only the lysozme offered positive staining in some cell of the bursa of Fabricius and thymus, despite non specific staining was observed in the other organs. In other studies, some of these antibodies have shown their efficiency in chicken (but in frozen tissues), and some in other species too. So there is no reason to give up on them as useful antibodies for this technique. Further research is needed.

Bibliography

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