New monoclonal antibodies (mAbs) of the 9th HLDA Workshop: an immunohistochemical analysis of the FACS and IHC panel mAbs on normal and activated skin and tonsil.

Venegoni L., Tavecchio S., Fiorani R., Fanoni D., Crosti C., Berti E.

Dept. of Dermatology Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico; "University of Milan-Bicocca. Milan, Italy.

Cross reactivity and multiple reactivities are very frequently detected in tissue studies; this study summarize the pattern of tissue staining of normal and pathological skin (affected from cutaneous lymphomas) and normal tonsil observed by using FACS and IHC panels of the 9th HLDA Workshop.

FACS panel: cryostat sections of frozen tissues were dried for 12 hours, fixed 10" in aceton and stained by an avidin-biotin immunoalkaline phosphatase method (DAKO). IHC panel: formalin fixed paraffin-embedded sections were dewaxed, heated in EDTA buffer (PH-8) for 15 min in a pressure cooker and immunostained by an avidin-biotin immunoalkaline-phosphatase method (DAKO). A) Skin reactivity results: N° 7 (FcRLB) and N°8 (CD20L) labelled epithelial cells (EP) and polymophonuclear leucocytes (PMN); N° 16 (XBP-1s) smooth muscle (SM), EP, dendritic cells (DR) and melanocytes (MEL); N° 18 DC, SM and EP; N° 20/21 (FCRL1-2) EP, collagen fibers (COLL) and nerves (N); N° 24 EP, COLL fibers, macrophages (MO) and fibroblasts (Fb); N° 26 (SC3) hair and eccrine gland (ADN); N° 30(CD210) EP; N° 32(CDW329) DR, MO, EP; N° 33 DC, EP and ADN; N° 34 MO, EP and AND; N° 35 MO; N° 39 (CD124) EP; N° 40(CD200) vessels (Vs) and ADN; N° 41(CD130) Vs and EP; N° 48(CD196) EP and ADN; 49(CD267) ADN; N° 52(CD191) EP and AND; 53(Int.beta5) and 54 (CD320) Vs, EP, AND, Fb; N° 55(DR4), N° 56(CMKLR1) EP and AND; N° 57(DR5) MO, Fb, EP, and AND; N° 58(Notch-1) Vs, EP and AND; N° 59 (4IgB7-H3) EP, Vs, striated muscle; N° 74(Int.beta6) EP and AND; N° 75(Dectin-1) Vs, EP and AND; N° 76-101(TREM-1) Vs, EP, MO and PMN; N° 77(Siglec-5/14) PMN and MO; N° 78(AMICA) Epand AND; N° 80(Siglec-9) MO, DR and PMN; N° 82(int.alfaVbeta5) MO, DR, EP, COLL, Fb, Vs; N° 84(DCIR) PMN; N° 85/99(GITR) PMN, EP and AND; N° 86(CD229) MO; N° 87(CD319) MO and plasmocytes (PC); N° 88(CD48) MO, DR and EP; N° 84(CD84) MO, DR and basal membrane (BM); N° 93(NTBA) MO, DR and PC; N° 94(HVEM) MO, Vs and EP; N° 95(TSLPR) nerves (Nv), EP and ADN; N° 98 (Galectin-3/Mac-2) MO, PMN, TBM, VS,COLL and EP; N° 100(Lymphotoxin betaR) EP; N° 106(CD150) MO-DR; N° 115(BLK 10B/2) Vs; N° 116(Bclx/8H) SM, EP, ADN, COLL, Fb; N° 128(CD38) EP; N° 223(AID) EP, ADN and MEL; N° 263(EVI2) MO and PC.

B) Tonsil reactivity with mononuclear cells and resident cells were detected in all mAbs, also if some reagents showed in our method high background (n° 6, 16, 17, 19, 24, 26, 27, 59, 69, 72, 81, 115, 116) or weak staining (n° 7, 8, 9, 10, 29, 32, 62, 67, 71, 78, 90, 91, 92). Results: N° 11(TRAIL-R2/DR5), 223 (AID), 263 (EVI2b) and 268 (LSP1) showed a pan leukocyte reaction; interestingly BLIMP1 specific N° 6/19 showed a cytoplasmic wide reactivity with FOL, PC, MO and T cell areas, whereas N°103/105 showed a strong nuclear staining of the PC and of the EP cells; N°13/107(BTLA) and 48(CD196-CCR6) stained PC and MG/MT zone of the FOL; N°

12/37/66 (PD1) and CD152 intrafollicular activated cells (IAC), whereas N°50(CXCR5) stained IAC and MG and MT zone of the FOL; N° 14(GCET-1), 17(LMO2), 18 (KLHL-6), N°70 (B7H4) stained GC; N° 29 GC and DR; N° 20/108(FCRL1), 21/110(FCRL2) MG/MT/GC and PC; N° 94 (HVEM), 114 (BLK154), 235 (BAFF-R) MG/MT zone; N° 97 (TCL1) nucleus of the FOL/MT zone; N° 27 (weak), 128-CD38 PC. We hope that our efforts may be useful to better understanding the tissue distribution of the analyzed reagents.