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Research paper

Detecting antibodies with similar reactivity patterns in the HLDA8 blind panel of flow cytometry data

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Abstract

The blind panel collected for the 8th Human Leucocyte Differentiation Antigens Workshop (HLDA8; http://www.hlda8.org) included 49 antibodies of known CD specificities and 76 antibodies of unknown specificity. We have identified groups of antibodies showing similar patterns of reactivity that need to be investigated by biochemical methods to evaluate whether the antibodies within these groups are reacting with the same molecule. Our approach to data analysis was based on the work of Salganik et al. (in press) [Salganik, M.P., Milford E.L., Hardie D.L., Shaw, S., Wand, M.P., in press. Classifying antibodies using flow cytometry data: class prediction and class discovery. Biometrical Journal].

Keywords: Antibodies; Classification; Cluster of differentiation; CD; Flow cytometry; Blind panel; Reactivity pattern; Kernel smoothing

1. Introduction

In the early HLDA workshops (Bernard et al., 1984; Reinherz et al., 1986; McMichael et al., 1987; Knapp et al., 1989; Schlossman et al., 1995; Kishimoto et al., 1997), proving that two independent antibodies bound to the same antigen with a novel molecular structure was both sufficient and necessary to designate a new CD specificity. Panels of flow cytometry data (conventionally called "blind panels") have been used to characterize the reactivity pattern of antibodies across cell populations of different lineages. It was shown that similarity in the reactivity patterns of newly discovered antibodies was a strong predictor of

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identity between their antigens. Blind panels were used to identify the groups of antibodies showing similar patterns of reactivity. Potential identity of specificity within these groups of antibodies was then evaluated by biochemical and molecular biological techniques, resulting in the assignment of a CD number to the antigen and the use of the same CD number to describe antibodies that exhibited specificity to that antigen. However, antigen cloning has rendered obsolete the requirement of having at least two independent antibodies of the same specificity to establish a new CD molecule. It is now considered appropriate (Mason et al., 2002) to establish a CD designation for a molecule if its gene has been cloned and at least one specific monoclonal antibody has been studied in the workshop. The role of flow cytometry data in the process of discovering new CDs has therefore decreased. Nevertheless, detecting similarity in the reactivity patterns of antibodies across a broad range of cell populations may still lead to the identification of new CDs and to assigning new antibodies to known CD clusters. This follows in part from the difficulty and unpredictability of molecular analysis, as it is more economically attractive to only invest in such analyses (Western blotting, immunoprecipitation) when several antibodies have the same apparent specificity. Furthermore, an analysis of antibody reactivity with antigenic post-translational variants (e.g. carbohydrate epitopes) provides a necessary and useful complement to biochemical and molecular analysis.

Biochemical and molecular characterization of antigens provides valuable background information, but users of antibodies against CD molecules are primarily interested in their reactivity with cells and tissues, which may vary in different applications. Flow-cytometric analysis of antibody reactivity ceased to be a major tool for discovering new molecules, but it continues to be central to HLDA workshop analysis and biological investigation.

This paper describes the application of a recently developed statistical methodology (Salganik et al., in press) to detecting groups of antibodies of similar reactivity in a panel of flow cytometry data collected by HLDA8 participating laboratories.

The development of statistical methodology for the design and analysis of blind panel experiments attracted only limited attention from researchers (e.g. Spiegelhalter and Gilks, 1987; Gilks et al., 1989; Gilks and Shaw, 1995; Hallam et al., 1997; Salganik et al., in press). Our approach to the analysis of the blind panel data is similar in spirit but differs in important implementation details (see also Salganik et al., in press) from the approaches used by Shaw et al. (1995) and Hallam et al. (1997). The novelty of our approach is that an automatic algorithm preselects a small subset of antibody pairs with "unusually high" similarity scores for subsequent visual inspection of the paired fluorescence staining profiles by a data analyst. This approach is similar to that used when searching for information on the Internet, where rapid search engines help focus the user's attention on the subset of possibly relevant objects. Previously used algorithms relied on summaries of the fluorescence intensity distributions (e.g. means and percentages of values above the threshold) in their evaluation of similarity between the antibodies. The matrix of similarity scores was used for hierarchical clustering of antibodies, and the summary of the resulting clusters was displayed in the form of a dendrogram. A data analyst used the dendrogram to identify antibodies that were clustered together and may therefore have identical specificity. However, the usefulness of this approach is bounded by the well-known deficiencies of hierarchical clustering. The structure of a dendrogram is often too sensitive to the intercluster similarity definition (i.e. the choice between group average, nearest neighbor and further neighbor methods) and small changes in the data. In addition, it is often too difficult to identify clusters based on visual inspection of the dendrogram or even to estimate the number of clusters in a dataset. Furthermore, important information may be lost when the distribution of log-fluorescence is summarized by the mean or mean and standard deviation. The partially automated approach to the detection of similarity in reactivity patterns, described herein, overcomes some of these limitations.

The analysis of the similarities in the staining patterns of HLDA8 antibodies guided the subsequent immunohistochemical and Western blot experiments as described by Swart et al. (2005).

2. Materials and methods

The HLDA8 blind panel of flow cytometry data included 49 antibodies of known CD specificity, 76