Antinuclear, Antinucleolar, and Anticytoplasmic Antibodies in Patients with Malignant Melanoma

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ABSTRACT

Immunofluorescence was used to examine antibodies to cellular antigens in the sera of patients with malignant melanoma. Sera from 60 melanoma patients and from 33 control individuals (13 normal subjects and 20 disease controls) were studied. Ninety % of the melanoma sera were found to have antinuclear antibodies when epithelial cell lines or melanoma lines were used as substrates for their detection, compared to 18% in the control group. Antinucleolar antibodies and anticytoplasmic antibodies were present in 32 and 17%, respectively, in malignant melanoma but none in the controls. Antinuclear and antinucleolar antibodies could be classified into different types according to different patterns of staining and susceptibility of antigens to digestion with DNase, RNase, and trypsin. Certain types of antibodies, such as those showing granular nuclear staining, appeared to be associated with less advanced stages of malignant melanoma, whereas those showing nucleolar and large speckled nuclear staining were associated with more advanced stages of the disease.

INTRODUCTION

The presence in MM patients of antibodies directed against membrane and/or cytoplasmic antigens of tumor cells has been firmly established (11, 17). Moreover, some activity against nuclear (16) and/or nucleolar (12) antigens has been found in these sera. A relationship between the presence of antibody against a particular antigen and the spread of the disease has been observed (10, 12, 22).

Recent studies concerned with the identification of autoantibodies to cellular antigens have demonstrated clearly that different tissue substrates including tissue sections and cell lines may be needed for detecting certain types of antinuclear antibodies. Treatment of tissues with fixatives also determines whether certain antigens are preserved or destroyed by such treatment. Examples of such features include the demonstration that proliferating cell nuclear antigen appears to be present only in proliferating cell lines (14) and that the Ku antigen associated with scleroderma-polymyositis is present in human and rabbit tissues but not in rat tissues (13). Certain cellular organelles, such as nucleoli and kinetochores, are larger and more distinct in tissue culture cells than in cells of highly differentiated tissues, making the former substrate more appropriate for identification of the corresponding antibodies. These observations resulted in the recognition of the high frequency of antinucleolar antibodies in scleroderma (26) and antikinetochore antibodies in the CREST subset of scleroderma (15).

With these recent observations as the rationale, this study was initiated to reexamine the occurrence of antibodies to cellular antigens in MM, using as substrates both tissue sections and different lines of tissue culture cells. A high occurrence of antibodies was observed in MM, and they were reactive with multiple nuclear and nucleolar components and with at least one cytoplasmic component. There appeared to be associations between certain types of autoantibodies and stages of malignant melanoma.

MATERIALS AND METHODS

Sera. Serum samples were collected from 60 patients with biopsy-proven MM with varying degrees of tumor spread and 33 control sera. Control sera were from 13 normal subjects, one patient with chronic osteomyelitis, one patient with a RBC antibody of unknown type, one patient with polycythemia vera, and 17 patients with a hematological or non-MM solid tumor (7 chronic myelogenous leukemia, 2 acute myelogenous leukemia, 1 undifferentiated “handmirror” cell leukemia, 2 lymphoma, and 1 each of lymphosarcoma, ovarian carcinoma, oat cell carcinoma, uterine carcinoma, and breast carcinoma). Sera were collected prior to the institution of therapy and were frozen in aliquots at −70°. Care was taken to avoid repeated freezing and thawing of serum specimens for the studies described below.

Immunofluorescence. Tissue sections and all cell smears were processed according to indirect immunofluorescence procedures described previously (24, 26). The sera and fluorescein-labeled immunoglobulin class-specific antisera (anti-IgG, anti-IgM, anti-IgA; Tago, Burlingame, Calif.) were diluted in PBS. Cell smears were usually counterstained for 30 sec with 0.05% Evans blue in PBS.

Substrates. Substrates used for indirect immunofluorescence studies consisted of snap-frozen 5.0-μm mouse kidney or human nevus sections and tissue culture cell lines. The human nevus was a pigmented marginal nevus obtained by punch biopsy from a healthy adult. The 2 cell lines used were human B-lymphoid cell lines [Ramos (8) and Wil2 (9)]; 2 cell lines were derived from human epithelial cancers [KB (5) and HeLa (6)]; and 2 cell lines were human melanoma lines [RPMI 8372 (21) and Colo 324 (22)] kindly provided by Dr. George Moore, Division of Surgical Oncology, Denver General Hospital, Denver, Colo.). HeLa, KB, RPMI 8372, and Colo 324 were maintained in Auto-Pow monolayer culture media (Flow Laboratories, Burlingame, Calif.) and the Wil2 and Ramos cell lines in Auto-Pow suspension culture media supplemented with 2 mm glutamine, vitamins, sodium pyruvate, non-essential amino acids, penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% heat-inactivated fetal calf serum (Flow Laboratories). Ramos and Wil2 were grown on a gyratory shaker. All the cells were seeded at a concentration of 2.0 × 105/ml and subcultured every 3 to...
4 days. Cells were harvested by centrifugation at 1000 rpm for 5 min, the monolayer cells being first detached by trypsin treatment. All cells were washed twice in PBS. Cell smears were prepared by cytocentrifugation and immediately fixed in acetone for 4 min at room temperature. Other fixatives such as methanol and ethanol were also examined, but acetone proved to be the best fixative for these studies.

**Mitotic Cells.** Mitotic cells were obtained from KB cells for analysis of staining patterns produced by antibodies reacting with chromosomes. Colcemid (0.20 μg/ml) (Grand Island Biological Co., Buffalo, N. Y.) was added to the culture medium of KB cells, and after 5 hr of incubation the cells were gently agitated to detach the mitotic cells. These detached cells were harvested by centrifugation, washed in PBS, and incubated in 0.075 M KCl at room temperature for 20 min. Cells were sedimented onto slides by cytocentrifugation and immediately fixed in acetone for 4 min.

**Enzymatic Digestion of Cell Smears.** Information concerning the nature of the cellular antigens reactive with antibodies was obtained by pretreatment of cell smears with various enzymes. DNase I, RNase A, and trypsin were obtained from Worthington Biochemical Corp. (Freehold, N. J.). The concentrations of enzyme used were 200, 100, and 50 μg/ml for DNase I and RNase A and 10, 6, and 3 μg/ml for trypsin. All were diluted with PBS with the exception of DNase I which was diluted with balanced salt solution containing 136 mM NaCl, 5 mM KCl, 1.5 mM KH₂PO₄, 1 mM CaCl₂, and 6 mM MgSO₄, pH 7.4. After fixation in acetone, cells were incubated for 10 min with the diluted enzymes at room temperature for trypsin and at 37° for DNase I and RNase A. The slides were washed twice in PBS prior to use as substrate for antinuclear antibody testing. Control slides without enzyme treatment were always tested, and the quality and specificity of the digestions were always confirmed with reference sera (anti-Sm, anti-ribonucleoprotein, anti-DNA). Anti-Sm staining was abolished by pretreatment of tissue with trypsin; anti-ribonucleoprotein staining was abolished with either trypsin or RNase A, and anti-DNA staining was abolished with DNase I.

**Anti-DNA Assay.** Antibodies to double-stranded DNA were measured by a Millipore filter technique (Millipore Corp., Bedford, Mass.) (7, 20). Antibodies to double-stranded DNA were considered significant if the binding was greater than 10%.

**RESULTS**

**Patterns of Nuclear Staining Produced by Melanoma Sera.** On KB cell smears, melanoma sera gave 4 major patterns of nuclear staining (Fig. 1) in the immunofluorescence assay using fluorescein-conjugated anti-IgG. The first pattern (Fig. 1A) has been designated "granular." This pattern of staining was present in both the nucleus and the cytoplasm. As shown in Fig. 1, the intensity of staining tended to vary from cell to cell on the same smear. This pattern was associated with a diffuse granular staining of chromosomes in mitotic cells (Fig. 1A, arrow). Thirty-five % of MM sera showed this pattern of staining.

The second pattern, designated "nodular," resembles the staining patterns reported previously on melanoma cells (Fig. 1B) (10, 16). In this pattern, nucleolar staining was present and was associated with clumpy "nodular" nuclear staining. This pattern was not associated with chromosomal staining in dividing cells. Forty-two % of MM sera stained KB cells in this pattern.

The third pattern was "patchy" staining (Fig. 1C). Patchy staining tended to be lumpy or densely speckled, and its distribution was both nuclear and cytoplasmic. In addition to morphological differences, it could be differentiated from the granular pattern by the absence of chromosomal staining in dividing cells. Twenty-two % of all MM sera tested showed this pattern of staining.

The fourth pattern called "large-speckled" (Fig. 1D) was somewhat similar to the anti-kinetochore staining described in the CREST subset of scleroderma patients (15, 26). Fig. 2 illustrates these 2 patterns of staining on KB cells. Both the large speckled pattern (Fig. 2A) and the kinetochore pattern (Fig. 2B) consisted of discrete speckles in the nucleus. One difference is illustrated in Fig. 2B (arrow) in which anti-kinetochore staining is associated with condensed chromosomes in dividing cells and persists as discrete speckles. With melanoma sera, the large speckled staining became diffuse in dividing cells. This is best illustrated on chromosome spreads in which the antigen was observed as diffuse staining around the periphery of the chromosomes (Fig. 2C), while anti-kinetochore sera demonstrated staining localized as discrete speckles at the centromeric regions of each chromosome (Fig. 2D). The large speckled pattern of nuclear staining was the most common pattern of staining seen with MM sera and was present in 58%.

All patterns were resistant to acetone fixation, although the intensity of patchy and nodular staining patterns began to decrease after 4 min of fixation. All nuclear staining except granular was removed by 5-min fixation in ethanol or methanol.

All these antinuclear antibodies and the observed frequencies were related to IgG antibodies. Only 2 sera were positive for IgM antinuclear antibodies and 3 for IgA. These 5 stainings were all of the granular pattern.

**Nucleolar Staining.** Distinct from the nodular pattern in which nucleolar staining was found in conjunction with the nodular nuclear staining as described above, 3 distinct patterns of nucleolar staining were also identified on KB cells. As illustrated in Fig. 3, some sera showed dense staining of the nucleolus (Fig. 3A), others demonstrated immunofluorescence localized to the perimeter of the nucleolus, designated a "ringed" pattern (Fig. 3B), and a few sera gave a speckled nucleolar pattern (Fig. 3C). The speckled nucleolar pattern was seen only in sera which also gave a large speckled nuclear staining. It is possible that some forms of nuclear staining may have obscured nucleolar staining. For this reason, we are unable to state with certainty the frequency with which these patterns coexist. All nucleolar staining patterns were observed with anti-IgG serum. Nucleolar staining with anti-IgA was found in 2 sera and with IgM in one sera. In some cases, the nodular pattern of nuclear staining was found associated with higher titers of nucleolar staining. Nucleolar staining was particularly susceptible to fixation with ethanol or methanol and was observed only after acetone fixation.

**Cytoplasmic Staining.** One MM serum showed immunofluorescent staining of the cytoplasm alone. Staining of the cytoplasm was usually seen in conjunction with other patterns of nuclear or nucleolar staining (Fig. 4). This staining was of variable intensity among different cells on the same smear but usually stained all cells to some degree. The cytoplasmic staining was typically speckled. The class of antibody giving cytoplasmic staining was most frequently IgG. Only 3 sera gave cytoplasmic staining with anti-IgM antisera.

**Frequency and Titer of Staining Pattern.** Most MM sera contained more than one antibody that reacted with cell nuclei, with nucleoli, or with cytoplasm, as demonstrated by overlapping patterns of staining. Patterns were sufficiently distinct; thus, they could be detected despite the presence of additional

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**Melanoma and Antinuclear Antibodies**

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1373

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antibodies. In the 60 MM sera tested, 1 antibody was detected in 12 sera, 2 antibodies in 21 sera, 3 antibodies in 14 sera, 4 antibodies in 8 sera, and no antibodies in 5 sera. No clear-cut association between different patterns of staining was found.

Table 1 shows the frequencies and titers of antibodies to cellular antigens in MM sera. Fifty-five of 60 (90%) of melanoma sera were positive versus 6 of 33 (18%) of control sera. The patterns observed in the control group included nuclear homogenous staining with sera from a patient with polycythemia vera and a patient with chronic myelogenous leukemia, antibody to centromere in a patient with breast carcinoma and a patient with RBC antibody, patchy nuclear staining in a patient with chronic myelogenous leukemia, and a nodular pattern in one normal subject.

The large speckled pattern, the most frequent (58%) pattern seen with MM sera, was the pattern showing highest titers. Granular staining (35%) was intermediate in titer. The nodular pattern (42%) and patchy staining (22%) were present mostly at low serum dilutions.

Nineteen MM sera showed distinct nucleolar staining (32%). As shown in Table 1, a number of antinucleolar antibodies were present in moderately high titer. Fifteen sera gave a dense nucleolar pattern, 2 sera gave a ring pattern, and 2 sera gave a speckled pattern. No nucleolar staining was found in the sera from the control groups.

The granular and patchy patterns of nuclear staining were regularly associated with cytoplasmic staining. Excluding sera with antibodies giving these 2 patterns, 10 additional sera were positive for anticytoplasmic antibodies. This cytoplasmic staining was seen alone or in conjunction with large speckled, nodular, or nucleolar staining. As seen in Table 1, antibodies giving cytoplasmic staining were usually present in low titer.

No anticytoplasmic reactivity was found in control sera.

Substrate Differences in Nuclear Staining Patterns. To clarify the role of different substrates in the detection of these antinuclear, antinucleolar, and anticytoplasmic antibodies, the indirect immunofluorescence assay was performed with 5 other cell lines in addition to KB cells and 2 tissue sections, human nevus and mouse kidney.

Table 2 summarizes the results obtained with the different substrates. All sera that gave granular staining on KB cells gave similar staining on all substrates tested. On tissue sections, the staining tended to be fine speckles rather than granular in pattern. The nuclear nodular pattern was positive on all the cell lines examined. On lymphoid cells (Ramos and WiL2), the observed pattern was homogeneous rather than nodular. The patchy staining pattern was seen on all cell lines but gave no staining on tissue sections. The large speckled staining pattern was restricted to KB, HeLa, and the melanoma lines but was not observed on human B-lymphoid cell lines or on mouse and human tissue sections. Cytoplasmic staining was demonstrated on all the epithelial cell lines (KB and HeLa) and melanoma lines but was absent on the lymphoid cell lines and on tissue sections. Nucleolar staining was found on all cell lines but was weakly positive on tissue sections.

Enzymatic Digestion of KB Cells. In studies characterizing other antibodies to nuclear antigens specifically autoantibodies seen in connective tissue diseases, it has been possible to identify the antibodies in precipitating systems (24). However, we were not successful in developing an immunodiffusion precipitating system for the antibodies found in MM sera. Several sources of nuclear antigens were tested: rabbit thymus extract (19) (Pel Freez, Rogers, Ark.); and soluble antigen extracts from WiL2, KB, and RPMI 8372 melanoma cells prepared in our laboratory according to techniques described previously (2). Representative sera were tested on multiple occasions with these extracts. Although precipitin lines were obtained with previously described antinuclear antibody systems, such as anti-Sm (19) and antiproliferating cells nuclear antigen (14), no precipitin lines were observed with MM sera. Therefore, antigen specificities were analyzed using a modification of the immunofluorescence technique in which substrates were pretreated with various enzymes. All patterns of nuclear staining on KB cells were removed after treatment of the cells with trypsin. A concentration of 1 µg/ml was adequate to obtain this result with all the patterns except the large speckled which required a higher concentration of enzyme (3 µg/ml). Cytoplasmic staining was also removed by trypsin (1 µg/ml) digestion, but nucleolar staining was not affected by this enzyme. Pretreatment of cells with DNase did not affect any of the staining patterns. The nucleolar reactivity of all sera but one disappeared after treatment of the cells with RNase A (50 µg/ml). The nuclear patchy pattern of staining and nuclear nodular staining were also removed by this concentration of RNase A.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>KB</th>
<th>HeLa</th>
<th>Melanoma line colo 324F</th>
<th>Melanoma line RPMI 8372</th>
<th>Ramos</th>
<th>Wil-2</th>
<th>Mouse kidney</th>
<th>Human nevus</th>
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<tr>
<td>Nuclear granular staining</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patchy staining</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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<td>0</td>
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<tr>
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<td>0</td>
<td>0</td>
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<tr>
<td>Cytoplasmic staining</td>
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<td>0</td>
<td>0</td>
<td>+</td>
<td>±</td>
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</table>

* Homogeneous nuclear staining observed instead of nodular.
Table 3
Relationship of antibodies to stages of melanoma

<table>
<thead>
<tr>
<th>Stage</th>
<th>Nuclear</th>
<th>Cytoplasmic</th>
<th>Large speckled</th>
<th>Nucleolar</th>
<th>Patchy</th>
<th>Nodular</th>
<th>Granular</th>
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<tr>
<td>I</td>
<td>37</td>
<td>16</td>
<td>18</td>
<td>50</td>
<td>10</td>
<td>26</td>
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<td>45</td>
<td>80</td>
<td>20</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>18</td>
<td>18</td>
<td>55</td>
<td>45</td>
<td>12</td>
<td>9</td>
</tr>
</tbody>
</table>

a The sum of the percentages of antibody staining for each disease stage exceeds 100% because of multiple antibodies present in individual sera.
b Information on the stage of melanoma was unavailable for one patient, and data on this serum are not included in this table.

table 4

Relationship of stage of MM to number of antibodies detected in patients' sera

<table>
<thead>
<tr>
<th>No. of antibodies</th>
<th>Stage I (n = 38)</th>
<th>Stage II (n = 10)</th>
<th>Stage III (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

a Information on the stage of melanoma was unavailable for one patient, and data on this patient are not included in this table.

Anti-DNA Activity of the Sera. No sera demonstrated significant anti-DNA activity in the Millipore filter radioimmunoassay. All binding values were less than 7% with a normal range in our laboratory of 0 to 10%.

Relationship of Autoantibodies to Stages of Melanoma. Table 3 gives the percentages of each autoantibody pattern found for the stage of the patient’s melanoma at the time of the serum collection. Stage I was MM localized to the primary site. Stage II was regional lymphatic dissemination of the disease, and Stage III was widespread disease (11). Granular staining was present mainly during Stages I and II of the disease, while an increase in the frequency of the large speckled pattern was observed during Stages II and III. In all stages, the frequency of patchy staining was similar. Nodular staining was found primarily in Stages I and III. Cytoplasmic staining was mainly present during Stages I and II of the disease, but nucleolar staining was found mainly during Stage III. No clear-cut differences in the distribution of the pattern were found with regard to Clark’s level of cutaneous invasion (4) or histological type of the melanoma. Also, there was no correlation between the number of antibodies detected in a patient’s serum and the stage of melanoma at the time the sample was collected (Table 4).

DISCUSSION

MM is considered to be one of the more immunologically active human tumors. The presence of antibodies against membrane or cytoplasmic antigens in the sera of MM patients has been documented extensively (1, 11, 17, 22, 27, 28). Several authors have reported the presence of nuclear (3, 10, 16) or nucleolar antibodies (12) in the sera of these patients. In this study, we extend the latter results. The nodular nuclear staining pattern described here might be similar to the pattern described by Morton et al. (16) and Lewis et al. (10) on MM cells. In addition, we report 3 distinct new patterns of nuclear staining, i.e., granular, patchy, and large speckled, and the different varieties of nucleolar staining. It was apparent in our studies that 2 factors might have been related to the detection of these new types of antinuclear and antinucleolar antibodies. Techniques of tissue fixation were extremely important, since fixation with ethanol and/or methanol tended to destroy the reactivity of the majority of antigens giving nuclear staining and all nucleolar antigens. Acetone fixation appeared to be most gentle, and even in this instance fixation times of longer than 4 min resulted in a progressive loss of antigenic reactivity. In addition, certain antibodies were detected using some tissue substrates and not others, and restriction of studies to a single tissue substrate may have resulted in a lack of detection of some antibodies.

As evidenced by the reactivity with different substrates (Table 2), it appears that the intracellular antigens recognized by antibodies in MM sera may be divided into 3 major categories. The first category of antigens appeared to be restricted to melanoma or epithelial cancer cell lines and were demonstrated as large speckled nuclear staining and cytoplasmic staining. The second category of antigens were present in all cell lines tested, including human B-lymphoid cells and were demonstrated as nuclear nodular and nuclear patchy staining. The third category was present in all tissues tested, including mouse kidney and human nevus sections and were demonstrated as nuclear granular and nucleolar staining. There could be several explanations for these findings including the hypothesis that “neoaotigens” were present in melanoma and epithelial cell lines and absent in lymphoid B-cells or organs, like kidney and nevus. Another equally tenable hypothesis is that certain cellular components were present in higher concentrations in undifferentiated or rapidly proliferating cells than in differentiated tissues and it was the quantity of this antigenic component which determined the sensitivity of the immunofluorescence technique. Our studies were not capable of differentiating between these 2 hypotheses. It is of interest that none of the antibodies were “melanoma specific” in that they were reactive only with 2 melanoma cell lines.

The characteristics of the 2 nuclear antigens demonstrating granular and large speckled staining are of interest in that, in dividing cells, they were in close association with the condensed chromosomes. The large speckled nuclear antigen, which in interphase cells showed speckled nuclear staining indistinguishable from anti-kinetochore staining, could be localized at the periphery of the daughter chromatids in striking contrast to kinetochore antigens, which were localized at the centromere region. The nature of the chromosome-associated large speckled antigen in MM would be of interest, since antibody was present in high frequency (58%) and high titer (Table 1), and it appeared to have some association with the more advanced stages of cancer.
The speckled cytoplasmic staining seen with some of the melanoma sera may be related to an antigen described recently by Natali et al. (18). These investigators described a cytoplasmic antigen present in human melanoma cells but not normal melanocytes, which reacted with the mouse monoclonal antibody 465.12 S against melanoma cells. This antigen could also be detected in normal epithelial cells of various organs especially of the colon, sigmoid flexure, and rectum and was found in cells from a large number of neoplasms. In most cases, the monoclonal antibody gave more intense immunofluorescent staining with tumor tissue than with normal cells of the same tissue origin. In our study, cytoplasmic staining was restricted to melanoma and epithelial cancer cell lines and was not found when lymphoid cell lines, mouse kidney, or human nevus were used as substrates.

The susceptibility of the different cellular antigens to enzymes serves in a general way to classify the antigen-antibody reactions. It should not be taken to signify that the antigens were protein, DNA, or RNA on the basis of their enzyme sensitivities. The antigenic determinants might be contained in multimolecular aggregates comprising nucleic acids and proteins, and it is possible that digestion of one component of an aggregate might disrupt the entire complex leading to loss of antigenicity. The proper identification of the chemical and molecular properties of the antigens was not within the scope of this study.

A relationship between the stage of melanoma and the presence of particular antibodies in the sera of MM patients has been demonstrated for antibodies to cytoplasmic antigens and membrane antigens (10, 11), and to nucleolar antigens (12). This report confirms previous studies showing a relationship between the presence of antibodies to cytoplasmic antigens and the early stages of MM (10, 11). The serological reactivity in this study, however, concerns only allogeneic anticytoplasmic antibodies since our sera were not from the same patients from whom the melanoma lines were developed.

In our study, antibodies to nucleolar antigens were found more frequently in sera from patients with late disease. McBride et al. (12) had reported previously that the presence of nucleolar antigens in melanoma biopsies from MM patients correlated with advanced clinical stages of the disease. Nucleolar antigens, which reacted either with autologous or allogeneic melanoma sera, were present in 45% of 140 melanoma biopsies tested and more commonly in biopsies from patients with late disease. McBride’s study, however, did not address directly the question of the frequency of antibodies to nucleolar antigens in melanoma sera. We detected antibodies to nucleolar antigens in 19 of 60 (32%) of melanoma sera tested. These antibodies reacted not only with allogeneic melanoma cells but also with epithelial cells, lymphoid cells, and in some cases with mouse kidney and human nevus sections. Antinucleolar antibodies were equally frequent in patients with Stage I and Stage II disease (26 and 30%, respectively) but were found more frequently (55%) in patients with Stage III disease. As far as antinuclear antibodies are concerned, a trend was noted in that granular staining antibody was more frequent in the early stages of MM and large speckled staining antibody in the later stages (Table 3).

The stimulus in malignant melanoma patients, which initiates the production of these antibodies, is obscure but some features are of interest. Unlike systemic lupus erythematosus, a disease characterized by multiple antibodies to cellular antigens including anti-DNA, the latter antibody was totally absent in MM sera. In addition, precipitating antibodies to many soluble nuclear antigens previously characterized in connective tissue diseases (24) were not detected in MM sera. Thus, it appears that the immune reactivity in MM is not a random immune response. The chemical and molecular nature of the nuclear, nucleolar, and cytoplasmic antigens identified with MM would be of interest in understanding the reasons for the selective immune response in this disease.

REFERENCES


Fig. 1. Melanoma sera were examined in the indirect immunofluorescence technique with KB cells as substrate. Some melanoma sera showed a granular pattern of nuclear and cytoplasmic fluorescence in dividing cells (A) associated with positive chromosomal fluorescence in dividing cells (arrow). A second group of sera demonstrated fluorescence in early and late S-phase with the fluorescence associated with specific nuclear fragments (B). A third pattern of fluorescence was consistent with normal nuclear fluorescence (C). A fourth pattern of fluorescence demonstrated by certain melanoma sera was large speckles localized to the nucleus (D). X 500.
Fig. 2. In the indirect immunofluorescence assay with KB cells as substrate, certain melanoma sera demonstrated a pattern of large speckles in the nucleus (A). × 600. This fluorescence pattern is similar to that which has been observed with scleroderma sera containing anti-kinetochore antibodies (B). × 600. The 2 types of staining could be easily distinguished by the localization of fluorescence on KB chromosomal spreads. The melanoma sera showed diffuse staining surrounding the chromosomes (C). × 1000, while scleroderma sera containing anti-kinetochore antibodies stained only the centromere portions of the chromosomes (D) × 600.
Fig. 3. Indirect immunofluorescence with KB cells as substrate. Melanoma sera gave several patterns of nucleolar fluorescence on KB cells: a clumpy nucleolar staining (A); fluorescence at the periphery of the nucleolus or "ring" pattern (B); and a speckled nucleolar staining (C). The latter nucleolar staining was associated with large nuclear speckles in the serum shown × 600.

Fig. 4. Indirect immunofluorescence with KB cells as substrate. Speckled cytoplasmic staining obtained on KB cells using a melanoma serum diluted 1:160. × 600.
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