Messy granuloma: an additional pattern of granulomatous reaction

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Abstract
Aim: There are six main types of granulomas in dermatopathology. The aim is to present and describe a seventh pattern of granuloma that we came across while studying biopsies of cutaneous leishmaniasis. Materials and Methods: We investigated 17 cases of cutaneous leishmaniasis. A granulomatous response was identified in 14 of them. The features that were common to all of them were noted. These common features were compared with the morphological characteristics of the already known six types of granuloma in 155 cutaneous biopsies from our archives. We also looked for figures of granulomas in cases of cutaneous leishmaniasis from the literature. Results: The granulomas identified in all of our cases of leishmaniasis were composed of epithelioid elongated glassy histiocytes, arranged in a randomly disorganized pattern. We dubbed these “messy” granulomas. Additionally, the granulomas presented with necrotic eosinophilic material that did not collect in the center, but rather intermingled with the elongated histiocytes. We found many examples in the literature in which the granulomas could be reclassified as messy. Conclusions: Messy granuloma – although not pathognomonic of leishmaniasis – seems to be quite characteristic of leishmaniasis, at least of cutaneous leishmaniasis.

Keywords: Leishmania, granuloma, tuberculosis, sarcoidosis, leishmaniasis.

Introduction
A granuloma is a compact collection of inflammatory cells in which mononuclear histiocytes predominate [1]. There are additional morphological variations, which are mainly determined by the type of histiocytes present in the granuloma and the architectural arrangement of these cells.

Six main types of common granulomas are distinguished (Figure 1).

Sarcoidal granulomas are usually composed only (or mainly) of epithelioid histiocytes, with no or very few other accompanying inflammatory cells. Epithelioid histiocytes are so-called because of their resemblance to epithelial cells, due to their wide, eosinophilic cytoplasm. Because of the absence of a peripheral shelter of lymphocytes covering the granuloma, sarcoidal granulomas are also sometimes referred to as “naked” granulomas.

In spite of the fact that the term sarcoidal is highly suggestive of sarcoidosis, sarcoidal granulomas are not specific to or pathognomonic of sarcoidosis or any other condition. They can indeed be seen in sarcoidosis, but they are also observed in some foreign body responses [2–4]. In fact, biopsies from cutaneous sarcoidosis show several types of granuloma other than sarcoidal granuloma in a relevant percentage of cases, including tuberculoid, supplicative, perineural, and foreign body granulomas [5].

Tuberculoid granulomas is the most common type of granuloma seen in cutaneous tuberculosis, with epithelioid histiocytes and Langhans’ giant cells [6]. These granulomas are often surrounded by a mantle of lymphocytes and some plasma cells. The center of the granuloma often appears occupied by caseifying necrotic acellular material. Again, tuberculoid granulomas are not pathognomonic of any condition, and they have been described in cases of granuloma annulare [7], in sarcoidosis [8, 9], lymphomas [10], and in several infectious diseases, such as leprosy [11].

Suppurative granulomas are composed of epithelioid histiocytes, some multinucleated histiocytes, and a center made up of a polymorphonuclear-rich inflammatory infiltrate. They are mainly associated with atypical mycobacteria [12] and fungal infections [13].

Foreign body granulomas show many angulated multinucleated histiocytes that surround the foreign bodies against which the granuloma has developed. The multinucleated cells often have nuclei arranged in a scattered patterned. However, some Langhans’ cells can also be seen [14].

Palisaded granulomas have epithelioid histiocytes arranged in a manner resembling stakes in a stockade. The center of the granuloma is often occupied by a substance that varies depending on the condition (mainly mucin in granuloma annulare, fibrin in rheumatoid nodules, or degenerated collagen in necrobiotic xanthogranulomas and in necrobiosis lipoidica) [15–17]. The granulomas described in recent years as being associated with some systemic conditions involving arthritis also belong to this category (e.g., palisaded neutrophilic granulomatous dermatitis with arthritis) [18, 19]. Palisaded granulomas have also been described in tattoos [17], in sarcoidosis [20], and in certain deep fungal infections [21].

Xanthogranulomas show – when fully developed – histiocytes with foamy cytoplasm, some of which can be multinucleated cells of the Touton type.
Figure 1 – Cutaneous granulomas. Schematic drawing of the six main types of granulomas that can be seen in dermatopathology (A–F; an approximate magnification of ×40) as well as of the messy granuloma presented in the current report (G). (A) Foreign body granuloma; (B) Palisaded granuloma; (C) Xanthogranuloma; (D) Sarcoidal granuloma; (E) Suppurative granuloma; (F) Tuberculoid granuloma.

We have seen a seventh type of granuloma when examining the inflammatory response seen in cutaneous leishmaniasis. It does not fit the description of the other six types of granuloma, and it shows certain morphological peculiarities, which we present in this report.

Materials and Methods

We studied the biopsies of 17 cases of cutaneous leishmaniasis. All of the cases were rich in amastigotes (the modified Ridley’s parasitic index [22] was ≥3 in all of them). The modified Ridley’s parasitic index is estimated from the number of amastigotes per standard section (6: ≥100,000; 5: ≥10,000; 4: ≥1000; 3: ≥100; 2: ≥10; 1: ≥1; 0: 0). In all cases, we evaluated the granulomatous response in terms of the morphology of the granulomas formed.

All cases were stained with Hematoxylin–Eosin (HE), Congo red, Masson’s trichrome, orcein, reticulin, periodic acid of Schiff (PAS), and Ziehl–Neelsen.

Additionally, we reviewed 155 skin biopsies of several types of granulomatous dermatitis not caused by Leishmania. In them, the granulomas had been categorized as one of the six common types of granuloma previously described in the literature. We excluded palisaded granulomas and xanthogranulomas; those types are peculiar enough as to not have any histopathological overlap with the type of granuloma discussed in the current report. We reviewed 21 cases of tuberculoid granuloma, 33 cases of suppurative granuloma, 63 cases of foreign body granuloma, and 38 cases of sarcoidal granuloma.

Results

In the group of cases of cutaneous leishmaniasis, we found a granulomatous response in 14 (82.35%) cases. In eight of them, this response was composed of discrete granulomas with well-demarcated borders. However, in six cases the granulomas were confluent, with poorly defined limits.

In all cases, the granulomas were composed of elongated epithelioid histiocytes arranged in a “messy”, “disorganized” or “pattern-less” pattern in which the long axes of the histiocytes seemed to be haphazardly arranged (Figures 1G and 2). In most of the cases, there were no multinucleated histiocytes, although such cells were found in four cases. These multinucleated histiocytes were not of the Langhans’ type (Figure 3A). We did not find asteroid or Schaumann bodies.

At a low magnification, the granuloma showed a loose appearance, with a low cellular density (Figure 3B). Two main phenomena contributed to this appearance. First, the epithelioid histiocytes showed a wide and glassy cytoplasm (Figure 3C). Second, a hyaline dense substance intermingled with the histiocytes (Figure 3E). This substance did not stain with Congo red, PAS, reticulin, or orcein. However, it presented a greenish color when stained with Masson’s trichrome (Figure 3F).

The “messy” granulomas were usually permeated by lymphocytes, which varied from discrete (eight cases), to moderate (three cases), and abundant (three cases) (Figure 3D). There was also a “crown” of lymphocytes surrounding the granuloma, i.e., they were not “naked” granulomas.

In the group of 155 cases of granulomatous dermatitis that we examined, we found 21 tuberculoid granulomas, 33 suppurative granulomas, 63 sarcoidal granulomas, and 38 foreign body granulomas. In this group, we did not find messy granulomas in any case. In other words, the granulomas did not meet the morphological criteria that we described for messy granuloma.

Among the tuberculoid granulomas, eight cases were from an unknown cause, seven cases were due to Mycobacterium tuberculosis infection, one case was an aluminum granuloma, one case was a cat scratch disease, three cases were chalazions, one case was due to an atypical mycobacteria infection, and one case was a granulomatous response against Demodex mites.

Tuberculoid granulomas did not show the low-cellularity appearance of messy granuloma when examined at a low power (Figure 4A). The cells were not arranged in a haphazard pattern. Necrosis was identified in many...
cases in the center of the granulomas, and Langhans’ cells were also a feature in many cases. The arrangement of the cells was especially polarized in the granulomas with central necrosis, although the latter was mild (Figure 4B). We saw one case of tuberculoid granuloma due to chalazions, which showed slightly elongated histiocytes vaguely evoking a messy granuloma (Figure 4C). However, the histiocytes were not as elongated as in messy granulomas, the characteristic eosinophilic substance intermingling with the histiocytes was not present, and some neutrophils were also found in the center of the granuloma (Figure 4D).

Among the sarcoidal granulomas, we found one case of Crohn’s disease, four silica granulomas, 27 chalazions, one foreign body reaction to coal particles, and 30 cases of cutaneous sarcoidosis.

Sarcoïdal granulomas did not show a haphazardly organized pattern. In contrast, a peculiar arrangement in nests or swirls was found to be characteristic of sarcoïdal granulomas (Figure 4, E and F). Moreover, fibrinoid necrosis was observed in the center of the granulomas in many cases (Figure 4G), and Langhans’ cells, asteroid bodies and Schaumann bodies were sometimes found (Figure 4H).

Among the suppurative granulomas, eight cases did not have a known cause, 10 cases were due to atypical mycobacterial infections, one case was a granulomatous reaction to a foreign body, four cases were chalazions, two cases were sporotrichosis, three cases were cryptococcosis, three cases were alternariosis, one case was an aspergillosis, and one case was a phaeohyphomycosis.

Among the 38 cases of foreign body granuloma, we could not identify the foreign body in 22 cases. Among the rest, the foreign bodies were keratin (eight cases), hair shafts (one case), suture thread (two cases), coal (one case), aluminum (one case), iron (two cases), and cholesterol (one case).

When we examined studies of cutaneous leishmaniasis in literature, in which pictures of the granulomatous response are shown, we found many examples in which the features of the granuloma meet the criteria described above for messy granuloma. For instance, in a very recent report on leishmaniasis by Kevric et al. [23], Figure 8 shows a perfect example of a messy granuloma.

Even cases with an unusual clinical presentation show the “messy” granulomatous pattern; for example, Bandyopadhyay & Bose reported a case presenting as rhinophyma [24]. Their Figure 3 shows this type of messy granuloma. Romero-Maté et al. presented two cases of cutaneous leishmaniasis in patients under treatment with Adalimumab [25]. Their patient 1 presented with a messy granulomatous response in the biopsy, which is shown in their Figure 2b. Giavedoni et al. presented a case of disseminated cutaneous leishmaniasis in a patient with Sézary syndrome [26]. Their Figure 2 is a high power view of the center of a granuloma in which the features of messy granuloma are easily identified. Youssef & Belhadjali reported a case of leishmaniasis that presented clinically imitating a necrobiosis lipoidica [27]. Their Figure 1B shows the characteristic messy granuloma.

Figure 2 – Messy granuloma: (A and C) Messy granuloma is made of epithelioid elongated glassy histiocytes, arranged in a random pattern; (B) In some cases, the granulomas were sharply demarcated from each other; (D) The histiocytes intermingle with a hyaline substance all over the granuloma, which in some cases appeared as thick collagen bundles. HE staining: (A and B) ×100; (C and D) ×200.
Figure 3 – “Messy” granuloma: (A) Giant cells were found only in occasional cases; (B) At a low-power magnification, the granuloma showed a low cellular density; (C) This visual effect was partly due to the wide glassy cytoplasms of the histiocytes; (D) In a few cases, lymphocytes were abundant in the granuloma, but the characteristic histopathological features described in the text were not obscured by the inflammation; (E) The histiocytes intermingled with a hyaline substance; (F) This substance had a greenish color when stained with Masson’s trichrome, and we inferred that it might be degenerated collagen (Masson’s trichrome staining, ×100). HE staining: (A) ×40; (B and D) ×100; (C and E) ×1000.

Figure 4 – Cutaneous granulomas: (A) Tuberculoid granuloma does not show as haphazard an arrangement as messy granuloma; (B) As soon as necrosis appears in the center of the granuloma, the histiocytes adopt a peculiar arrangement around the necrotic material. HE staining, ×100.
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Figure 4 (continued) – Cutaneous granulomas: (C) Some tuberculoid granulomas seen in chalazions have certain overlapping features with messy granuloma, such as elongated histiocytes arranged in a certain random pattern; (D) However, the intermingling with degenerated collagen was not found to be a feature of these tuberculoid granulomas; (E and F) Sarcoideal granuloma is not haphazardly arranged but shows a characteristic arrangement in swirls; (G) Fibrinoid necrosis is sometimes seen in the center of sarcoideal granulomas; (H) Schaumann bodies are a feature of sarcoideal granuloma but not of messy granuloma. HE staining: (C and E) ×25; (F) ×40; (D) ×100; (G) ×200; (H) ×400.

In some other cases, the features of the messy granuloma are identifiable even though the lesion is highly infected with amastigotes to the point of obscuring the other histopathological characteristics of the lesion. For instance, this is seen in Figure 2 of the case by Newlove et al. [28]. In some other instances, the granuloma appears infiltrated by many lymphocytes, but still the messy arrangement of the elongated epithelioid histiocytes is easily identified. An example of this is seen in Figure 1A of the paper by Paniz Mondolfi et al. in a case of infection by *L. infantum* [29].

Discussion

Granulomas are the result of a complex cellular response to the interaction between antigens or irritants and inflammatory cells and biological mediators. Under the influx of inflammatory substances, the histiocytes adopt an epithelioid appearance – sometimes fusing to form multinucleated cells – and organize in a compact pattern that is called a granuloma. Granulomas can be classified according to different criteria, which vary from etiopathogenic to morphological. The latter distinguishes six main types of cutaneous granulomas: tuberculoid, sarcoideal, suppurative, foreign body, palisaded, and xanthogranulomas. Granulomatous response in leishmaniasis varies with the amount of amastigotes present in the organism. It has been claimed that individualized granulomas are usually associated with a small amount of microorganisms [23]. Such a granulomatous response in leishmaniasis has been
described in the literature as belonging to the sarcoidal, foreign body, tuberculoid, or palisaded types [23]. However, when we examined multiple cases of cutaneous leishmaniasis, as well as pictures from the literature, we were able to easily identify a curious reproducible pattern that did not seem to fit any of the previously described six types of granuloma. In this pattern, the histiocytes appear elongated, and their main axes are arranged in a randomly irregular pattern. We have dubbed this type of “messy” granuloma due to the disorganized arrangement of the elongated histiocytes. Moreover, the histiocytes are accompanied by a type of hyaline substance that does not collect in the center of the granuloma, but rather intermingles with the histiocytic population.

The cell-poor loose pattern seen in messy granuloma when examined at a low magnification is mainly due to two reasons: the glassy appearance of the cytoplasm of the histiocytes and the eosinophilic substance intermingling with the epitheloid histiocytes.

Suppurative granulomas and foreign body granulomas are very different from messy granulomas. The first group, by definition, shows central containment of neutrophils. All of the other features of messy granuloma are lacking. Foreign body granulomas do not show any of the features of messy granuloma either. Moreover, the multinucleated angulated histiocytes—which often surround the foreign bodies—are characteristic of foreign body granuloma.

We performed several histochemical techniques in an attempt to identify this hyaline substance. The laces of the hyaline substance were only stained in a greenish color by Masson’s trichrome. This indicates that they are probably degenerated collagen. “Messy” granuloma is probably not pathognomonic of leishmaniasis, in spite of the fact that we failed to find it in any other granulomatous condition. We saw one case of tuberculoid granuloma in a chalazion that showed certain morphological features evoking messy granuloma. Specifically, this granuloma showed a certain elongation and disorganization of the histiocytes. However, in messy granuloma, the elongation is in general more pronounced. Moreover, no cases of chalazion presented the intermingling degenerated collagen seen in messy granuloma.

Messy granuloma is not the only granulomatous reaction seen in cutaneous leishmaniasis. Although none of our cases presented with any other type of granuloma, clear cases of tuberculoid granulomas associated with cutaneous leishmaniasis have been presented in the literature [30]. In fact, in a study of 317 patients with cutaneous leishmaniasis, the authors reported granulomas in 61.5% of the cases, of which 25.2% were caseating granulomas, 4.6% were suppurative, and 70.2% were tuberculoid [31]. However, it would be interesting to see how many of these granulomas would be reclassified as messy granuloma [25, 29]. However, we also found potential examples of messy granuloma in cases of infection by *L. donovani*.

Conclusions

We have identified a type of granuloma with characteristic histopathological features in cases of cutaneous leishmaniasis. The main feature of this type of granuloma is elongated epithelioid histiocytes with a glassy cytoplasm, arranged in a random pattern and intermingling with degenerated collagen. We have dubbed this reaction “messy” granuloma. Although it is probably not pathognomonic of leishmaniasis, we failed to identify it in any other cutaneous condition.

Conflict of interests

The authors declare that they have no conflict of interests.

References

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